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Ameliorative influence of *Ginkgo biloba* extract on acetaminophen-induced oxidative stress in livers and kidneys of male albino rats

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Abstract

In this study, the effects of *Ginko biloba* extract on -treated groups were divided subchronically-induced toxicity of acetaminophen (AAP) on functions of livers and kidneys of male albino rats were investigated. Subchronically into 8 groups. Animals of group1 received tap water daily for 1 week and served as controls. Animals of group 2 were treated with *Ginko biloba* extract (GBE) (50 mg /kg b.wt). Animals of group 3 were treated daily for 1 week with AAP (250 mg/kg b. wt.). Animals of group 4 were treated daily for 1 week with GBE (50 mg /kg b.wt) and AAP (250 mg/kg b. wt.). As for groups 5, 6, 7 and 8, they were treated experimentally as groups 1, 2, 3 and 4 but for 2 weeks. Determination of both liver and kidney functions was used as early indicators for the detection of liver damage and for evaluation of renal toxicity after exposure to the test articles. Meanwhile, serum concentration of malonedialdehyde was measured to assess the deleterious oxidative influence of AAP on the liver and kidneys. Also, the antioxidant glutathione (GSH) concentration and activities of antioxidant enzymes, glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD), were assayed under the present experimental conditions. The data demonstrated a significant increase in serum liver function enzymes such as aminotransferases (ALT and AST) as well as alkaline phosphatase subsequent to administration of AAP, whereas a significant decrease in GSH content and in the activities of GPx, GST, CAT, and SOD was observed after 1 and 2 weeks of treatments with this test article. The results also indicated that administration of 50 mg GBE/kg b. wt. was enough for nearly normalization of various parameters examined, which appears to be due to the protective effects of GBE on AAP-induced oxidative stress in the liver and kidneys.

Keywords: Acetaminophen, *Ginko biloba,* kidney, liver

1 Introduction

Recently, a considerable body of work has been published about herb-drug interactions, including several comprehensive reviews (Cup, 1999; Loannides, 2002 and Ulbricht, et al., 2006). Interest in this topic is easily understood given the growing popularity of alternative medicine, the myriad botanicals available worldwide as dietary supplements, the multitude of unique phytochemicals present in these products and the paucity of knowledge regarding their pharmacology. More importantly, almost 25% of all prescription drug users take herbal medicines concomitantly with conventional medications (Wold, et al., 2005 and Gardiner, et al., 2006). Acetaminophen or paracetamol is a widely used over the counter analgesic (pain reliever) and antipyretic. It is commonly used for the relief of headaches, other minor aches and pains, and is a major ingredient numerous cold and flu remedies. Also acetaminophen can be used as an alleviative care in advanced cancer patients; therefore, acetaminophen is very important drug, although it leads to many problems when taken in overdose and for a long time. Moreover, recent studies have shown that many plants used in traditional medical practices have antioxidant properties. From this point, extracts of *Ginkgo biloba*

are used in herbal medicine for the treatment of mild to moderate cognitive *disorders* (Mahadevan and Park, 2008). The aim of this work was to assess the beneficial effects of *Ginkgo biloba* in alleviating the toxic effects of acetaminophen particularly on the liver and kidneys.

2 Materials and Methods

Animals: The present experiments were performed using male albino rats weighing about 100-120 g. The animals were randomly segregated into the following groups:

Animals of group1 received tap water daily for 1 week and served as controls. Animals of group 2 were treated with *Ginko biloba* extract (GBE) (50 mg /kg b.wt). Animals of group 3 were treated daily for 1 week with AAP (250 mg/kg b. wt.). Animals of group 4 were treated daily for 1 week with GBE (50 mg /kg b.wt) and AAP (250 mg/kg b. wt.). As for groups 5, 6, 7 and 8, they were treated experimentally as groups 1, 2, 3 and 4 but for 2 weeks. Blood samples were collected after one and 2 weeks of treatments where they were received from the retro-orbital plexus veins by a fine capillary glass tube into clean centrifuge tubes and left to clot at room temperature. They were centrifuged at 3000 rpm for 10 min and the supernatant serum samples were then dispensed into Epindorff tubes and kept frozen at -20 ^{o}C until analysis.

Biochemical analyses:

Liver function:

Alanine aminotransferase enzyme (ALT) was assayed according to Gella, et al. (1985) whereas aspartate aminotranseferase (AST) was measured colorimetrically according to the method of Reitman and Frankel, (1957). As for Alkaline phosphatase (ALP) levels, it was determined according to Burstein, et al. (1970).

Kidney function:

Determination of urea was based upon the cleavage of urea with urease according to the method of Fawcett and Scott (1960). Determination of uric acid was undertaken according to the method of Barham, (1972) and serum creatinine was determined according to the method of Doolan et al. (1962).

Determination of antioxidant factors:

Glutathione peroxidase (GPx) was determined according to the method of Paglia and Valentine (1990). Determination of catalase (CAT) activity was assyed according to the method of Aebi (1974). Superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972). Determination of glutathione (GSH) was achieved by enzymatic colorimetric method according to Beutler et al. (1963). Glutathione -S- transferase (GST) in the liver tissue of the tested animals was determined according to the method of Habig and Jakoby, (1981). Determination of lipid peroxidation, a major indicator of oxidative stresses, was determined by measuring malondialdehyde (MDA) in the liver according to the method of Ohkawa et al. (1979).

Statistics:

All data were expressed as means±SD. Groups of data were compared with an analysis of variance (ANOVA) followed by multiple comparison tests, values of $P < 0.05$ were regarded as significant.

3 Results

ALT, AST and ALP levels in serum were significantly higher in the AAP group when compared with those of control group. Although, extracted *Ginkgo biloba* (EGB) treatment decreased these values after one and two weeks of administration as seen in table (1). Table (2) showed that after one and two weeks of administration, serum urea levels were significantly increased in acetaminophen groups (42.36 ± 0.15) in comparison with control group (26.23 ± 0.94), also, it has been found that, serum uric acid levels were significantly increased in AAP group (2.01 ± 0.23) in comparison with control group (1.02 ± 0.02). Serum creatinine (mg/dl) after one week of administration was significantly increased in AAP group (1.05± 0.05) in comparison with values of the control group (0.39 ± 0.02). Table (3) illustrates that serum glutathione peroxidase activity was significantly decreased in acetaminophen groups (27.54 ± 0.26) compared with its control group value (44.80±1.63). Glutathione levels (mg/dl) decreased significantly in AAP group (18.26 ± 0.28), also, it has been found that glutathione decrease in EGB group but did not show any significant difference. Catalase activity decreased in AAP group as well as in AAP +

EGB group but did not show any significant difference in the later group when compared with control group. Table (4) displays that liver glutathione -S- transferase activity (nmol/mg protein/min) significantly decreased in AAP group (413±128), whereas its value has been improved in AAP + EGB group (692±131) after 2 weeks of treatment, but did not show any significant difference when compared with the value of the control group (655±121). Likewise, similar pattern of changes has been observed concerning liver glutathione.

Table (1). Changes of serum ALT, AST and ALP as affected by acetaminophen and /or *Ginkgo biloba* treatments in male albino rats:

	The 1st week of treatment			
	Control	EGB	AAP	EGB+AAP
**ALT	19.26±0.82	20.51±0.26	40.54±1.04*	25.19±1.72
**AST	39.62±0.54	33.71±1.57	66.16±1.76*	42.13±1.07
**ALP	91.58±2.36	91.26±1.54	134.27±5.34*	98.16±2.03
	The 2nd week of treatment			
	Control	EGB	AAP	EGB+AAP
**ALT	20.03±0.26	25.16±0.74	46.25±0.87*	32.56±0.7
**AST	38.57±1.26	32.54±1.76	75.16±5.26*	48.37±1.26
**ALP	94.34±1.26	95.30±1.87	146.26±5.48*	102.23±5.30

*P < 0.05; compared to control group. **IU/L
For each group, n=5.
Results are expressed as means ± SD.

Table (2). Changes of serum urea, uric acid and creatinine as affected by acetaminophen and /or *Ginkgo biloba* treatments in male albino rats:

	The 1st week of treatment			
	Control	EGB	AAP	EGB+AAP
**Urea	26.23±0.94	21.01±0.72	42.36±1.15*	25.64±0.84
**Uric acid	1.02±0.02	0.82±0.01	2.01±1.23*	1.26±0.01
**Creatinine	0.39±0.02	0.40±0.08	1.05±0.05*	0.40±0.03
	The 2nd week of treatment			
	Control	EGB	AAP	EGB+AAP
** Urea	26.15±1.04	22.16±0.02	49.79±1.56*	29.52±1.34
** Uric acid	1.23±0.23	1.26±0.25	3.56±0.13*	1.54±0.97
**Creatinine	0.38±0.21	0.38±0.05	1.12±0.02*	0.43±0.51

**mg/dl

Table (3). Changes on serum GSH, GPx, CAT and SOD as affected by acetaminophen and /or *Ginkgo biloba* treatments in male albino rat:

	The 1st week of treatment			
	Control	EGB	AAP	EGB+AAP
** GSH	32.54±1.24	36.21±1.20	18.26±0.28*	28.64±1.52
*** GPx	44.80±1.63	46.82±1.62	27.54±0.26*	39.62±1.04
****CAT	12.26±2.15	11.23±1.26	7.92±0.49*	10.28±1.26
**SOD	165.46±0.45	169.95±3.31	130.90±2.35*	150.41±2.56
	The 2nd week of treatment			
	Control	EGB	AAP	EGB+AAP
** GSH	32. 52±1.25	34.20±1.94	17.51±0.12*	21.25±0.15
*** GPx	45.62±1.41	42.51±0.20	21. 62±0.71*	18.25±0.46
****CAT	12.54±1.59	13.26±1.20	7.46±0.28*	11.81±1.02
**SOD	166.25±3.71	197.23±2.15	135.24±2.47*	149.25±3.10

**u/ml, ***mg/dl, ****µmol/sec/ml

Table (4). Changes of liver Glutathion -S- transferase and glutathione as affected by acetaminophen and /or *Ginkgo biloba* treatments in male albino rats.

	The 1st week of treatment			
	Control	EGB	AAP	EGB+AAP
*** GST	682±1.32	842±1.62	413±1.28*	694±1.20
** GSH	7.52±0.44	7.02±0.314	3.43±0.529*	3.23±0.232*
	The 2nd week of treatment			
	Control	EGB	AAP	EGB+AAP
*** GST	655±1.21	821±1.29	420±1.18*	692±1.31
** GSH	8.91±0.74	6.21±0.43	4.23 ±0.23*	6.63±0.04

µg / mg protein. * n mol/mg protein/min.

Liver superoxide dismutase activity (u/mg protein) was found to decrease in acetaminophen group in comparison with control whereas glutathione peroxidase (n mol /min/mg protein) showed nearly the same pattern of change. On the other hand, *Ginkgo biloba* treatment didn't cause any significant change in comparison with control group. Also, the combination of *Ginkgo biloba* and acetaminophen didn't cause any significant change in superoxide dismutase or in glutathione peroxidase activity when compared with their comparable control values (Fig. 1&2).

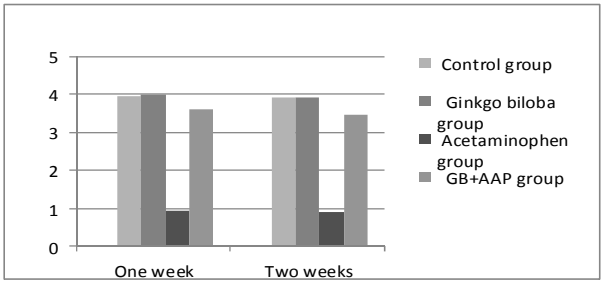

Fig (1): SOD (U/mg protein) level in liver of control group, *Ginkgo biloba* group (EGB), acetaminophen group and in AAP + EGB group. For each group n=5, P>0.001 verses control group.

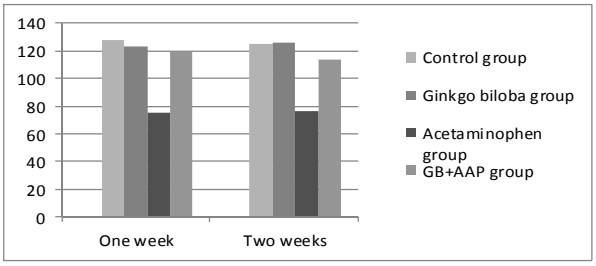

Fig (2): GPx (n mol/min/mg protein) level in liver of control group, *Ginkgo biloba* group (EGB) acetaminophen group and in AAP + EGB group.

Liver CAT (u/mg protein) activities were significantly decreased by the treatments with acetaminophen (Fig. 3) after 1 and 2 weeks of treatments compared with control values.

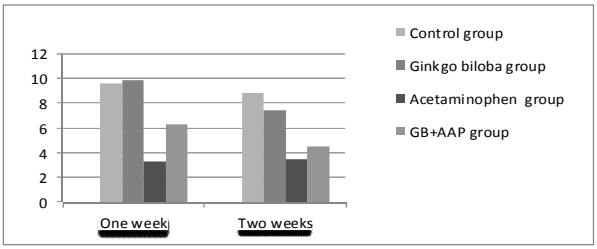

Fig (3): CAT level in liver of control group, *Ginkgo biloba* group (EGB) acetaminophen (AAP) group and (AAP + EGB).

Liver MDA (m mol/mg protein) increased significantly in AAP group when compared with the value of the control group (Fig 4) after 1 and 2 weeks of treatments.

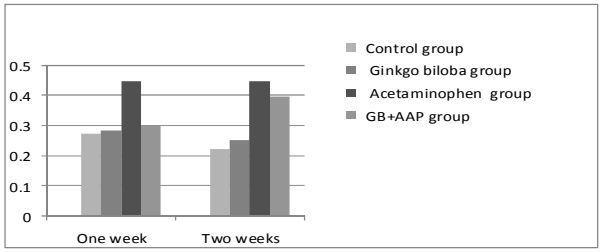

Fig: (4): MDA level in liver of control group, *Ginkgo biloba* group (EGB), acetaminophen (AAP), and (AAP + EGB) group.

4 Discussion

In the current study, significant elevations in serum ALT, AST and ALP levels were observed after ip. administration of 250 mg/kg b. wt. of acetaminophen (AAP) for 7 and 14 days in comparison with their respective control groups. These results are consistent with previous findings (Sallie, et al., 1991)**.** On the other hand, co-administration of both *Ginkgo biloba* extract (GBE) and AAP helps in decreasing effects of AAP-induced toxicity and this effect was clear in the decrease of serum ALT in GBE + AAP group in comparison with AAP group. Serum ALT and AST measure the concentration of intracellular hepatic enzymes that have leaked into the blood circulation and serve as markers of hepatocytes injury. The increased activity of ALT, AST and ALP in AAP groups appeared to be related to the damage occurred in the liver cells as reflexed by change in hepatic function markers. Accordingly, the changes in levels of these serum enzymes have been attributed to the damaged structural integrity of the liver because these enzymes are normally located in the cytoplasm of the hepatocytes and are released into the circulation after hepatic damage (Sallie, et al., 1991). The biochemical alteration in the liver function enzymes showed that high doses of AAP when co-administrated with GBE seemed to decrease AAP-hepatotoxicity, suggesting that GBE may have a property of hepato-protective influence. In a trial for interpretation of the changes in serum enzymes suggesting hepatic injury, serum malodialdehyde (MDA) was measured and was found to increase after daily AAP administration, particularly after 14 days of exposure. This finding apparently refers to oxidative damage induced by AAP particularly in the liver cells. This suggestion is corroborated by the fact that the antioxidant glutathione (GSH) concentration in liver and activities of hepatocyte antioxidant enzymes, glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD), decreased due to AAP treatment. On the other hand, administration of GBE prevented the decrease in these antioxidants to nearly the normal levels. The biochemical markers such as urea, uric acid and creatinine are usually used to evaluate renal function. Creatinine is defined as an amino acid derivative with a molecular mass that is freely filtered by the renal glomeruli. Because of creatinine is freely filtered by the kidneys and is not reabsorbed, creatinine clearance can be used as an indicator of kidney function. Administration of 250 mg/kg b. wt. AAP for 7 and 14 days causes a significant rise in serum urea, uric acid and creatinine levels which was consistent with findings of other authors (Lim, et al., 1995). The creatinine excretion is dependent almost on the process of glomerular filtration, thus, the significant rise in the serum creatinine level might be due to the impairment of glomerular function and tubular damage in the kidney as suggested recently by Mansour and Mossa (2010). However, other previous studies did not found a significant rise in plasma creatinine level after AAP administration (Futter et al., 2001, perrone et al., 1992 and pakravan et al., 2007).The protective effect of GBE against AAP toxicity was not as obvious in kidneys as it was in case of the liver. This may be due to additional mechanisms contributing to AAP toxicity in kidneys (Van Erp et al., 2009). Different mechanisms and sensitivity of renal tissue (von mach et al., 2005) or different in situ biotransformation might have influence in this context (Sakuma et al., 2009). The MDA and GSH levels as well as GSH, GPx, CAT and SOD activities in the liver are shown in table (4) and Figs. 1, 2, 3 and 4. The MDA levels in the liver of animals that were administered AAP alone were observed to display significant increase compared with control group and this increase was attenuated by co-administration of GBE. Also, significantly reduced levels of GSH and GPx, GST, and SOD activates were seen in the liver tissues of AAP-treated rats compared with their control animals.

In the present study, SOD decreased in AAP group but increased in AAP and GBE group. In previous studies, the coordinate action of antioxidant system is very critical for the detoxification of free radicals. SOD reduced the concentration of highly reactive superoxide radical by converting it to H_2O_2 whereas catalase and GPx decomposes H_2O_2 and protects the tissue from highly reactive hydroxyl radicals (Sahreen et al., 2001). Acetaminophen produces a decrease in

serum catalase activity. However, the co-administration of GBE and AAP resulted in rise in activity of catalase in serum. The cumulative oxidative damage is likely one of the mechanisms producing the hepatotoxic effects of acetaminophen administration (Ekam and Ebong, 2007). Also, it has been found that blood glutathione peroxidase decreased in animals of AAP group in comparison with their controls. The co-administration of AAP and GBE produces a subtle decrease in serum glutathione peroxidase activity as well as in glutathione content indicating the ameliorative influence exerted by GBE against AAP toxic effects. In conclusion, the present results suggest the beneficial influence exerted by GBE in protection of liver and kidneys against the free radicals and peroxidative influence produced as a result of acetaminophen consumption in large doses for long periods.

5 References

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