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Protective Effects of Ginkgo biloba on Acrylamide -Induced Rats Hepatotoxicity

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ABSTRACT

Background: Hepatotoxicity is the term used to describe harm or liver damage brought on by drug use or other nonpharmacological factors. White crystal chemical acrylamide (ACR) is a typical raw material for polyacrylamide products. Traditional Chinese medicine called Effect of Ginkgo Biloba (EGb) comprises flavonoids and other active ingredients with significant medicinal qualities. Administration of EGb repaired the harm caused by ACR. The protective effect of EGb is achieved by encouraging neuronal regeneration. It is commonly known that EGb works very well to increase antioxidants and blood flow to the brain.

Objectives: The aim of the current work was to assess by histological and biochemical tests the potential protective impact of Ginkgo biloba extract against the hepatotoxicity caused by acrylamide.

Materials and methods: Forty Wister male albino rats of the local strain of average body weight 160±15 g were used in this study. The experimental healthy animals were housed in separate appropriate cages with a 12-hour light/dark cycle in an environmentally controlled breeding room at a temperature of 22°C and a humidity of 60%, with free access to food and water. Rats were fed a standard diet of processed rat food and water. Liver enzymes and Oxidative stress indicators were measured.

Results: Alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase levels were significantly higher in the acrylamide-treated group compared to the untreated, ginkgo biloba-treated, and acrylamide + ginkgo biloba-treated groups (P<0.001). Malondialdehyde levels were also considerably higher in the acrylamide-treated group (20.021.91) compared to the untreated control group (5.220.66), the ginkgo biloba-treated group (4.590.35), and the acrylamide + ginkgo biloba-treated group (8.411.24) (P<0.001).

Conclusion: It could be concluded that the hepatoprotective of Ginkgo Biloba action serves as a preventative and therapeutic measure. An aqueous extract of G. biloba leaf contains a variety of chemical components that may function as a mechanism to reduce acute liver damage by scavenging oxidative free radicals, inhibiting lipid peroxidation, and possessing antioxidant activity.

Keywords: Acrylamide, Ginkgo biloba, Hepatotoxicity, Rats.

INTRODUCTION

In the paper and cosmetics industries, in gel electrophoresis, and as a soil stabilizer, acrylamide is a chemical that is employed **[1].** There are several various ways to be exposed to acrylamide, including smoking cigarettes, eating potato chips and sweets, and drinking water that has been flocculent treated **[2].**

High-temperature roasted food frequently contains the pollutant acrylamide (ACR) **[3].** It has been demonstrated that the production of acrylamide during heating is temperature dependent. Starchy meals that had been heated above 120°C, such as bread, French fries, and potato chips, were found to contain acrylamide. It was not discovered in meals that had been boiled or not cooked. It addressed items like cookies, crackers, and morning cereals that are frequently consumed over time **[4].** Acrylamide's potential biological effects have received a great deal of interest when it was discovered that some cooked meals contain it in 2002, and this has highlighted the need for additional research into its neurotoxicity, hepatotoxicity, and reproductive damage **[4].**

Acrylamide (ACR) exerts its toxic effects by promoting the production of reactive oxygen species, which causes oxidative damage and weakens antioxidant defence mechanisms, leading to multiorgan failure at large dosages **[2].**

Previous investigations showed that ACRL causes liver cell damage in experimental animals **[1].** It lowers the quantity of hepatic protein by allowing protein reserves from the hepatocytes to seep out **[2].** Fortunately, antioxidants help shield liver tissue from the damaging effects of pollutants and other medications. Due to their availability as natural sources of antioxidants, plants have long been regarded as one of the principal treatments for poisoned livers **[5].**

Ginkgo biloba (GB), one of the oldest medicinal tree species, protects against poisons brought on by radiation as well as chemical and natural toxins. Overall, research shown that GB has antioxidant, anti-inflammatory, anti-apoptotic, and antigenotoxic actions in various toxicities **[6].**

According to **Sener *et al.* [7],** who showed that the oxidative damage caused by mercury in the liver was reversed by the antioxidant properties of Ginkgo biloba **[8],** Ginkgo biloba extract decreased ALT and AST liver enzyme levels and improved hepatocyte edema and necrosis.

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The aim of the current work was to assess by histological and biochemical tests the potential protective impact of Ginkgo biloba extract against the hepatotoxicity caused by acrylamide.

MATERIALS AND METHODS

***Chemicals*:** Acrylamide (ACR) from Sigma Chemical Company was made just before usage by dissolving it in distilled water [9]. It was purchased by Amriya Company (Egypt) for Pharmaceutical Industries under license from Beaufour Ipsen International, Paris, France [10]**.** GB extract was used as Tanakan 761 (EGB).

Animals:

Forty Wister male albino rats of the local strain of average body weight 160±15 g were used in this study. They were purchased from Assiut University's Experimental Animal House (Egypt). The experimental healthy animals were housed in separate appropriate cages with a 12-hour light/dark cycle in an environmentally controlled breeding room at a temperature of 22°C and a humidity of 60%, with free access to food and water. Rats were fed a standard diet of processed rat food and water. Before the trial began in their new setting at the Toxicology Department, Faculty of Medicine, Al-Azhar University, Assuit, the rats were kept for two weeks to acclimate.

Experimental design

The rats were divided randomly into four groups (n=10).

- **Group I (normal control):** rats received 5 ml of distilled water daily by oral gavage for 6 weeks (no treatment).
- **Group II (Ginkgo biloba treated group):** rats received 0.1 ml of water containing Ginkgo biloba extract by oral gavage at a dose level of 40 mg/kg body weight [11]**,** daily for 6 weeks.
- **Group III (Acrylamide treated group):** rats received Acrylamide by oral gavage.
- **Group IV (Acrylamide + ginkgo biloba treated group):** rats received Acrylamide and ginkgo biloba.

Collection of blood samples

At the conclusion of the experiment, rats were anaesthetized, put to sleep, and the chest was opened. Blood samples were taken from the heart and placed in plain and citrate containing tubes. Sera and plasma were separated and kept in Eppendorf tubes. They were then stored refrigerated at -20^{o}C until biochemical analyses.

Biochemical study:

Alanine aminotransferase enzyme (ALT) was identified as having liver functions by **Gella *et al.*** [12] Alkaline phosphatase (ALP) levels were assessed using the Reitman and Frankel method**,** aspartate aminotransferase (AST) using the Burstein, *et al.* method [13, 14]. Oxidative stress indicators included malondialdehyde (MDA) levels in plasma, reduced glutathione (GSH) levels, plasma catalase (CAT) activity, and plasma superoxide dismutase (SOD) activity. These measurements were done using the methods of **Chattopadhyay *et al.*** [15]**, Beulter** [16]**, Aebi** [17] and **Misra and Fridovich**[18]**.**

Ethical considerations:

This study was ethically approved by Ethical Committee, Faculty of Medicine, Al-Azhar University, Assuit. The National Institutes of Health (NIH) criteria for the care and handling of Mice used in research were followed. An organization in charge of animal ethics authorized all tests and ensured that they were conducted in accordance with The Clinical and Laboratory Standards Institute's (CLSI) recommendations for the treatment of animals and the proper disposal of their waste.

S*tatistical analysis*

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 24 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Information is presented as mean ± SD (standard deviation). One-way analysis of variance (ANOVA) and the Tukey post-hoc test was used to determine the statistical significance of differences between experimental groups. 0.05 was used as the P value for significance.

RESULTS

Forty Wister male albino rats of the local strain were examined, and it was found that the acrylamide-treated group had significantly higher levels of ALT, AST, and ALP than the normal control, ginkgo biloba-treated, and acrylamide + ginkgo biloba-treated groups (P 0.001), respectively **(Table 1).**

Table (1): Liver enzymes among the studied groups of rats

Variable	Mean ±SD	F	P value	95% CI	
				Lower	Upper
ALT (IU/L)					
Normal control group (N=10)	19.90±2.47	202.018	**<0.001***	18.13	21.67
Ginkgo biloba treated group (N=10)	21.10±3.45			18.63	23.57
Acrylamide treated group (N=10)	104.30±14.29			94.08	114.52
Acrylamide + ginkgo biloba treated group (N=10)	57.10±9.52			50.29	63.91
Post Hoc P (Mean Difference)	*P1=0.763 (1.20), P2<0.001*(84.40), P3<0.001*(37.20), P4<0.001*(83.20), P5<0.001*(36.00), P6<0.001*(47.20)*				
AST (IU/L)					
Normal control group (N=10)	35.10±4.01	256.924	**<0.001***	32.23	37.97
Ginkgo biloba treated group (N=10)	31.00±2.98			28.87	33.13
Acrylamide treated group (N=10)	90.80±6.89			85.87	95.73
Acrylamide + ginkgo biloba treated group (N=10)	57.70±6.67			52.93	62.47
Post Hoc P(Mean Difference)	*P1= 0.099(4.10), P2<0.001*(55.70), P3<0.001*(22.60), P4<0.001*(59.80), P5<0.001*(26.70), P6<0.001*(33.10)*				
ALP (IU/L)					
Normal control group (N=10)	93.30±4.40	312.213	**<0.001***	90.15	96.45
Ginkgo biloba treated group (N=10)	91.90±3.21			89.60	94.20
Acrylamide treated group (N=10)	162.70-8.00			156.98	168.42
Acrylamide + ginkgo biloba treated group (N=10)	118.90±6.82			114.02	123.78
Post Hoc P(Mean Difference)	*P1=0.600(1.40), P2<0.001*(69.40), P3<0.001*(25.60), P4<0.001*(70.80), P5<0.001*(27.00), P6<0.001*(43.80)*				

ALT: Alanine aminotransferase enzyme. **AST:** Aspartate aminotransferase. **ALP:** Alkaline phosphatase.
F: One way ANOVA test. *****Significant. **CI:** Confidence interval for Mean.
P1: normal control compared ginkgo biloba treated group.
P2: normal control compared acrylamide treated group.
P3: normal control compared acrylamide + ginkgo biloba treated group.
P4: ginkgo biloba treated group compared acrylamide treated group.
P5: ginkgo biloba treated group compared acrylamide + ginkgo biloba treated group.
P6: acrylamide treated group compared acrylamide + ginkgo biloba treated group.

Malondialdehyde levels were considerably higher in the acrylamide-treated group (20.02±1.91)) compared to the untreated control group (5.22±0.66) the ginkgo biloba-treated group (4.59±0.35) and the acrylamide + ginkgo biloba-treated group (8.41±1.24)(P<0.001) While the glutathione, catalase, and superoxide dismutase levels in the ginkgo biloba treatment group were considerably higher than those in the acrylamide treatment group and the acrylamide + ginkgo biloba treatment group(P<0.001) **(Table 2).**

Table (2): Oxidative stress indicators among the studied groups of rats

Variable	Mean ±SD	F	P value	95% CI	
				Lower	Upper
MDA (nmol/ml)					
Normal control group (N=10)	5.22±0.66	358.422	**<0.001***	4.75	5.69
Ginkgo biloba treated group (N=10)	4.59±0.35			4.34	4.84
Acrylamide treated group (N=10)	20.02±1.91			18.66	21.38
Acrylamide + ginkgo biloba treated group (N=10)	8.41±1.24			7.52	9.30
Post Hoc P(Mean Difference)	*P1=0.247(0.63), P2<0.001*(14.80), P3<0.001*(3.19), P4<0.001*(15.43), P5<0.001*(3.82), P6<0.001*(11.61)*				
GSH (u/ml)					
Normal control group (N=10)	32.10±2.39	86.014	**<0.001***	30.39	33.81
Ginkgo biloba treated group (N=10)	35.30±3.56			32.75	37.85
Acrylamide treated group (N=10)	18.40±2.59			16.55	20.25
Acrylamide + ginkgo biloba treated group (N=10)	22.90±1.91			21.53	24.27
Post Hoc P(Mean Difference)	*P1=0.011(3.20), P2<0.001*(13.70), P3<0.001*(9.20), P4<0.001*(16.90), P5<0.001*(12.40), P6=0.001(4.50)*				
CAT (µmol/sec/ml)					
Normal control group (N=10)	13.21±0.87	124.417	**<0.001***	12.59	13.83
Ginkgo biloba treated group (N=10)	14.77±1.17			13.94	15.60
Acrylamide treated group (N=10)	6.97±0.93			6.30	7.64
Acrylamide + ginkgo biloba treated group (N=10)	10.40±0.90			9.76	11.04
Post Hoc P(Mean Difference)	*P1=0.001(1.56), P2<0.001*(6.24), P3<0.001*(2.81), P4<0.001*(7.80), P5<0.001*(4.37), P6<0.001* (3.43)*				
SOD (u/ml)					
Normal control group (N=10)	168.70±5.91	162.037	**<0.001***	164.47	172.93
Ginkgo biloba treated group (N=10)	201.70±11.77			193.28	210.12
Acrylamide treated group (N=10)	128.00±7.35			122.74	133.26
Acrylamide + ginkgo biloba treated group (N=10)	144.20±5.16			140.51	147.89
Post Hoc P(Mean Difference)	*P1<0.001*(33.00), P2<0.001*(40.70), P3<0.001*(24.50), P4<0.001*(73.70), P5<0.001*(57.50), P6<0.001* (16.20)*				

MDA: Malondialdehyde. **GSH:** Glutathione. **CAT:** Catalase. **SOD:** Superoxide dismutase.
F: One way ANOVA test. *****Significant. **CI:** Confidence interval for Mean.
P1: normal control compared ginkgo biloba treated group.
P2: normal control compared acrylamide treated group.
P3: normal control compared acrylamide + ginkgo biloba treated group.
P4: ginkgo biloba treated group compared acrylamide treated group.
P5: ginkgo biloba treated group compared acrylamide + ginkgo biloba treated group.
P6: acrylamide treated group compared acrylamide + ginkgo biloba treated group.

DISCUSSION

Hepatotoxicity is the term used to describe harm or liver damage brought on by drug use or other nonpharmacological factors. It is an unfavorable pharmacological reaction that could be rare but serious, and as a result, have a significant negative effect on health [19]. White crystal chemical acrylamide (ACR) is a typical raw material for polyacrylamide products. ACR is utilized in numerous sectors all over the world for pulp processing, pipeline inside coating, and water filtration [20]. Furthermore, after being cooked at high temperatures (above 120°C), foods high in starch can result in ACR. Ataxia and skeletal muscular weakness are features of a neurological disorder caused by ACR. The main locations of ACR action are found in nerve terminals, according to quantitative morphometric and electrophysiological investigations [21]. Traditional Chinese medicine called Effect of Ginkgo Biloba (EGb) comprises flavonoids and other active ingredients with significant medicinal qualities. Neurodegenerative disorders and cerebral ischemia injury are protected against by EGb [22].

Therefore, the purpose of the study was to determine whether Ginkgo biloba extract would have some protective effects against the hepatotoxicity caused by acrylamide by histological and biochemical analyses. The Experimental Animal House of Assiut University provided forty Wister male albino rats of the local strain (body weight 160 ± 15 g) for use in the current investigation (Egypt).

In the current investigation, acrylamide-treated groups showed significantly higher levels of the enzymes alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase than normal control, ginkgo biloba-treated, and acrylamide + ginkgo biloba-treated groups (P<0.001). An open-field test and gait analysis were used in a different study by **Huang *et al.*** [23]. In line with a prior work, they stated that ACR-treated mice displayed walking tremors as well as weakness or paralysis in the rear limbs [24]. They also noticed that specific neurotoxicity symptoms, such altered behavior and aberrant gait, were produced in a unique way when ACR entered the body. However, not all of our experimental results support this supposition. Administration of EGb, however, repaired the harm caused by ACR. These findings support the idea that EGb promotes neuronal regeneration in order to have a protective impact. It is well known that EGb is particularly efficient on promoting cerebral blood circulation and antioxidants [25].

As carbon tetrachloride (CCl4) is one of the most hepatotoxic substances, **Khattab** [26] reported that GbE was tested for its hepatoprotective potential utilizing CCl4 produced acute hepatotoxicity in rats. The rats intoxicated with CCl4 also showed a substantial reduction in food intake, FER, and weight growth percentage when compared to the control group, according to the results. These findings were consistent with **Chang *et al.*** [27]. In comparison to the CCl4-impaired group, pretreatment with GbE in rats resulted in a significantly higher weight gain percentage, food intake, and FER. These results revealed that the administration of the extract considerably reduced the toxic effects of CCl4 and aided in hepatocyte regeneration. These findings were completely consistent with those of **Farooq *et al.*** [28]. While **Wen-yuan *et al.*** [29] observed that CCl4's effects were dramatically reduced after pretreatment with Ginkgo leaf extract. These findings demonstrate the effectiveness of GbE as a hepatoprotective drug against CCl4-induced liver damage. The most significant active components in G. biloba extract that have an antioxidant effect are terpenoids (ginkgolides and bilobalides) and flavonoids (ginkgo-flavone glycosides) [30]. This supports **Ding et al research** [31].

In this regard, **Abdul-Hamid *et al.* research's** [8] demonstrated that supplementing with grape seed or Ginkgo biloba significantly reduced blood ALT and AST levels after 8 weeks, demonstrating an improvement in enzyme function. The variations in the ALT and AST enzymes' activity were improved by ginkgo biloba. The study supports the findings of **Parimoo *et al.*** [32], who observed that Ginkgo biloba extract helped to bring back high serum ALT and AST levels to normal levels. Ginkgo biloba extract decreased DNA fragmentation % in the liver tissue of rats that had undergone pre- or post-c-irradiation [33], and decreased DNA damage from N-nitrosodiethylamine by decreasing several comet assay parameters [34].

Our findings showed that malondialdehyde levels were considerably higher in the acrylamide-treated group (20.02±1.91) compared to the untreated control group (5.22±0.66), the ginkgo biloba-treated group (4.59±0.35), and the acrylamide + ginkgo biloba-treated group (8.41±1.24) (P<0.001). As opposed to the normal control, acrylamide-treated, and acrylamide + ginkgo biloba-treated groups, glutathione, catalase, and superoxide dismutase levels were significantly higher in the ginkgo biloba treated group (P<0.001). **Gedik *et al.*** [9] earlier research showed that ACRL caused liver cell damage in test animals. Death and turnover of hepatic cells result from liver damage. Chronic damage causes ongoing cell loss and turnover, which triggers an ongoing inflammatory response, the activation of hepatic stellate cells (HSC), which transform into my fibroblasts, and tissue repair responses, which lead to fibrosis and eventually cirrhosis.

In regards to this issue, **Erkekoglu and Baydar** [35] have demonstrated that ACR generates equivalent neurotoxicity at low and high doses, with lesser doses merely necessitating a longer exposure time. Additionally, ACR-poisoned mice exhibit signs of peripheral and central nervous system injury [27]. ACR can result in distant axonal swellings and degeneration, pathological lesions, and changes of nerve terminals. ACR-mouse neurotoxicity is characterized by

significant gait impairments. More significantly, mice treated with ACR had reduced mobility and signs of paralysis in their hind limbs, which were unable to sustain their body weight. They suggested that ACR might damage the central nervous system by impairing synaptic transmission or, alternatively, by altering how the efferent system operates. Due to the lack of specialized medications to treat ACR toxicity, numerous studies have concentrated on the active components of herbal plants. Ginkgo biloba extract (EGb) exhibits preventive properties against cardiovascular disease and neuroplasticity. In order to treat ACR poisoning, they utilized EGb [30].

The impact of GbE on hepatic malondialdehyde (MDA) and reduced glutathione (GSH) in CCl4-intoxicated rats was also observed by **Khattab** [26]. The levels of MDA in the rats' liver tissue were substantially higher in the CCl4-intoxicated group than in the control group, according to the results. However, pretreatment of rats with GbE exhibited improvement in hepatic MDA content, as the value of MDA showed much lower as compared to the CCl4 group, while the results of the rats receiving GbE tended to match control value. In terms of hepatic GSH, the results showed that rats given CCl4 were significantly less affected than the control group. Rats given GbE as a pretreatment have significantly conserved hepatic GSH.

CONCLUSION

It could be concluded that the hepatoprotective of Ginkgo Biloba action serves as a preventative and therapeutic measure. An aqueous extract of G. biloba leaf contains a variety of chemical components that may function as a mechanism to reduce acute liver damage by scavenging oxidative free radicals, inhibiting lipid peroxidation, and possessing antioxidant activity. Given that GbE has been clinically prescribed for the treatment of many disorders, this absence of toxicity of GbE should be taken into consideration if safety precautions for public health are to be established in response to growing consumption of this herbal by human populations. Administration of EGb lessens the effects of ACR-induced neuronal damage, mostly through encouraging neuronal regeneration. Therefore, EGb counteracts the neurotoxicity caused by ACR.

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