Hepatoprotective effect of ginseng, green tea, cinnamon their combination against Acetamiprid - induced oxidative stress in rats

Abstract

The present study aimed to investigate whether ginseng, green tea and cinnamon or/and mixture can improve liver in an rats and whether this therapeutic approach resulted in amelioration in lipids profile (serum cholesterol, triglycerides, Serum HDL, Serum LDL, FT3 and FT4), liver function profile (serum aspartate transaminase, alanine transaminase, total protein and albumin) and cytokines profile (serum tumor necrosis factor-α, interleukin-1ß and interleukin-6). Male albino rats weighing 150 g ± 10% were used. They were divided into two groups: negative control and Acetamiprid groups. The results demonstrated that Acetamiprid induced antioxidant and biochemical alterations at tested periods 15 and 30 days. The obtained results revealed a significant (P<0.001) increase in Lipid parameters in Acetamiprid rats than those in their control ones. Lipid parameters (serum cholesterol, triglycerides, free fatty acids & cholesterol) were significantly (P<0.001) elevated in Acetamipired rats compared with their corresponding control group. On the other hand, induction of Acetamipired to rats caused a significant (P<0.001) decrease in adiponectin level.. Liver function tests (serum aspartate transaminase and alanine transaminase) were significantly (P<0.001) increased in Acetamipired rats compared with their corresponding control group But, the levels of serum total protein and albumin were remarkable decreased in Acetamipired rats group. A considerable (P<0.001) elevation were occurred in all cytokines parameters (serum tumor necrosis factor-α, interleukin-1ß, interleukin-2 and interleukin-14) in Acetamipired rats group compared with their corresponding control group. When, Acetamipired rats group was treated with ginseng, green tea and cinnamon or/and mixture a considerable amelioration effects in all previous studied parameters were pronounced dependent on certain mechanisms and time of treatment.

Introduction

Pesticides are the chemical formulation increasingly used in agriculture, animal husbandry and public health operation to kill the insects, weeds and fungus and to get rid of insect transmitted diseases. The frequent and continuous use of pesticide has resulted in their widespread distribution in the environment. These pesticides are toxic not only to insects and pests but at different levels to animals and human beings [1]. These agrochemicals, if not properly used, may pose serious hazards to human and animal health. Therefore, the present-day concern is with regard to their judicious and proper use, so that they can be applied safely with proper instructions and guidance to have minimum risk to human and animal health [2]. The neonicotinoids, the newest major class of insecticides, have outstanding potency and systemic action for crop protection against piercing- sucking pests [2] and they are highly effective for flea control on cats and dogs [3]. Several neonicotinoids are harmful to honeybees, either by direct contact or ingestion. This may lead to a reduction in pollination [4].

Acetamiprid (ACP) is very operative for aphids, leafhopper and pests of leafy vegetables. The selectivity of ACP is due to the electronegative nature of nitroguanidine,

nitromethylene and nitroamine which bind with an exact subsite in nAChRs. The metabolites could be considered as an inhibitor of inducible nitric oxide synthase [5].

Acetamiprid is a member of the neonicotinoid group of insecticides and it is the most highly effective and largest selling group of insecticides worldwide for crop protection. Also it used along to control fleas infesting in livestock and pet animals [6]. In addition, Acetamiprid is being highly water soluble indicates a high potential for the compound to leach in soil or to run off in surface water [7]. And, it may be reach to human or non-target organisms via the pesticide residues in air, water, and food chain [5]. So, more research about the side effect of Acetamiprid need to be available. On the other hand, the histopathology is a critical part of the toxicologic and risk assessment of foods, drugs, chemicals, biologics, and medical devices.

In recent years, the health benefits [8] of consuming green tea, including the prevention of cancer [9] and cardiovascular diseases [10] the anti-inflammatory [11], antiarthritic [12], antibacterial [13], antiangiogenic [14], antioxidative [15], antiviral [16], neuroprotective [17] and cholesterol-lowering effects [18] of green tea and isolated green tea constituents are under investigation. However, adding green tea to the diet may cause other serious health concerns.

The chemical composition of green tea is complex: proteins (15-20% dry weight), whose enzymes constitute an important fraction; amino acids (1-4% dry weight) such as theanine or 5-N-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, and lysine; carbohydrates (5-7% dry weight) such as cellulose, pectins, glucose, fructose, and sucrose; minerals and trace elements (5% dry weight) such as calcium, magnesium, chromium, manganese, iron, copper, zinc, molybdenum, selenium, sodium, phosphorus, cobalt, strontium, nickel, potassium, fluorine, and aluminum; and trace amounts of lipids (linoleic and a-linolenic acids), sterols (stigmasterol), vitamins (B, C, E), xanthic bases (caffeine, theophylline), pigments (chlorophyll, carotenoids), and volatile compounds (aldehydes, alcohols, esters, lactones, hydrocarbons). Due to the great importance of the mineral presence in tea, many studies have determined their levels in tea leaves and their infusions [19]. Fresh leaves contain, on average, 3-4% of alkaloids known as methylxanthines, such as caffeine, theobromine, and theophylline [20]. In addition, there are phenolic acids such as gallic acids and characteristic amino acid such as theanine present**.**

Ginseng is a widespread herbal medicine [21] and it has served as an important component of many Chinese prescriptions for thousands of years [22]. Today it still occupies a permanent and prominent position in the herbal (best-sellers) list and is considered the most widely taken herbal product in the world [23]. Moreover, it is estimated that more than six million Americans are regularly consuming ginseng products [24]. They do not only believe that ginseng will engender physical bene-fits, but that it will also have positive effect on their cognitive performance and well-being.

Cinnamon is a common spice used by different cultures around the world for several centuries. It is obtained from the inner bark of trees from the genus Cinnamomum, a tropical

evergreen plant, Cinnamon bark, leaves, flowers and fruits are used to prepare essential oils, which are destined for use in cosmetics or food products. Moreover, according to traditional Chinese medicine (dating roughly 4000 years), cinnamon has been used as a neuroprotective agent [25] and for the treatment of diabetes [26] has also been used as a health-promoting agent for the treatment of diseases such as inflammation, gastrointestinal disorders and urinary infections [27]. Another potential medical use of cinnamon would be with regards to its antimicrobial properties, especially antibacterial activity. It is well known that infection is one of the leading causes of morbidity and mortality worldwide. It is this chemical diversity that is likely to be the reason for the wide-variety of medicinal benefits observed with cinnamon. The aim of the present study was to investigate the effects of gensing, green tea and cinnamon extracts and their mixture on hematobiochemical alterations in male rat exposed to Acetamiprid.

Materials and Methods

Acetamiprid administration:

Acetamiprid (CAS No-135410-20-7) was purchased from Sigma Aldrich, USA. 100 mg acetamiprid administrated with the aid oro-gastric tube which dissolved in distilled water /kg of body weight/day for 6 weeks according to [28].

Medicinal herbs:

Three medicinal herbs were employed in this study. They were ginseng (*Ginseng Panax*) roots, green tea (*Camellia sinensis*) leaves and cinnamon (*Cinnamomum zeylanicum*) bark and obtained from local market (Harraz Herbs Market in Nasr City, Cairo, Egypt). The medicinal plants were grinded mechanically and sieved prior to their extraction.

Extracts preparation:

Aqueous hydro-distilled extracts of ginseng (*Ginseng Panax*) roots, green tea (*Camellia sinensis*) leaves and cinnamon (*Cinnamomum zeylanicum*) bark were prepared by simple distillation. In brief; 5 g of dried fine powder of each herb was boiled in 100 ml deionized distilled water (5%) for 15 minutes and left until cooled then the extractions were filtered through Milipore 0.2 microns filter prior to use. The aqueous filtrated extractions were preserved in dark bottle and stored at 4◦C.

Animals:

A total of 70 healthy male albino rats (Rattus rattus) weighing 150 g ± 10% were used throughout the whole study. The animals were obtained from the laboratory animal house of the Nile Pharmaceutical Company, Cairo, Egypt. The experiments were conducted according to the ethical norms approved by Ethics Committee of Faculty of Science, Ain Shams University, Egypt. Animals were kept under full hygienic conditions, an ambient temperature of 25 ± 2°C and 12/12 hours of light–dark cycle, had free access to fresh water and fresh well-balanced diet, and kept under supervision for two weeks before commencing the experimental work. The animals were housed in all groups of five rats per cage. The rats were received Acetamiprid (ACP) insecticide orally (drinking) at dose 100 mg/kg body weight (1/10 oral LD_{50}) dissolved in tap water every day for 30 days [29].

Experimental design

Rats were randomly divided into two main groups (Control and Acetamiprid) which were further subdivided into subgroups. Acetamiprid group consists of 5 subgroups were used

in repeated treatments respectively. The first subgroup was kept as a recovery. The four subgroups were treated orally by ginseng, green tea, cinnamon and mixture for 15 and 30 days.

Blood collection and tissue preparation

At the end of each experimental period, rats were deprived of food over-night, anesthetized with diethyl ether and dissected. Blood samples were collected from each group in clean dry test tubes, left to clot at room temperature then centrifuged at 3000 r.p.m for 15 minutes for separation of serum. Sera were kept at -20 °C until the determination of biochemical parameters.

The serum parameters were analyzed spectrophotometrically by using double beam UV-Visible spectrophotometer (VIS-JR, model 1601). Estimation of cholesterol (enzymatic method), triglyceride (enzymatic method) and HDL-cholesterol (phosphotungstate method) were carried out using respective diagnostic kits (Randox, Ltd., Co., UK). LDL-cholesterol was calculated as per Friedewald´s equation [30].

Serum FT3, FT4 and insulin were assayed by coated -A- count FT3, FT4 or insulin kits utilizing a solid phase radioimmunoassay technique and 125 I as a tracer (Diagnostic Product Corporation, Los Angles, USA). Plasma ADMA and serum total nitric oxide and endothelin-1 were assayed by ELISA (Sandwich Immunoassay Technique) using commercial kits (Assay Designs, Inc-Germany).

In liver, thiobarbituric acid reactive substance (TBARS) was measured using commercial kits purchased from Cayman Chemical Company (Ann Arbor, USA.). The estimation of glutathione (GSH) and glutathione peroxidase (Gpx) levels were estimated

tumor necrosis factor-α, interleukin-1ß, interleukin-4 and interleukin-12 levels were assayed using commercial ELISA (Sandwich Immunoassay Technique) specific kit for rats (Diagnostic Automation, INC. USA) [31].

Statistical analysis:

Results were expressed as the mean ± standard error (SE) for all animals in each group. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests according to [32] and [33]. Results were considered significantly different if were $P < 0.001$ and $P < 0.05$.

RESULTS

The daily oral dose of ACP (100 mg/kg) for two and four weeks showed a significant increase ($P < 0.05$) in the enzymatic activities of serum AST and ALT. In addition, the serum levels of TNF-α, IL-1β, IL- 12 and IL- 4 showed a significant increase ($P < 0.05$) in ACP group

compared with control group, indicating that the hepatotoxicity was induced by ACP. Moreover, depletion of liver GSH was found in rats treated with ACP compared with control ($P < 0.05$). Liver TBARS was measured as a marker of lipid peroxidation. It was found that liver TBARS was significantly elevated in rats treated with ACP compared to control ($P < 0.05$). All adverse alterations increased aggressively with time (Table 1).

Table (1): A comparison between normal and acetamiprid exposure rats groups on lipid, liver function, cytokines and tissues liver profiles (Mean ± SE).

Parameters	Groups	Control n = 10	Acetamiprid n = 10
Lipid Profile	**Serum cholesterol** (mg/dL)	55.873 ± 0.839	109.407 ± 1.478*
	Serum triglycerides (mg/dL)	64.136 ± 0.928	137.841 ± 1.749*
	Serum HDL-Ch (mg/dL)	14.553 ± 0.021	21.718 ± 0.056*
	Serum LDL-Ch (mg/dL	28.493 ± 0.032	60.121 ± 0.098*
	FT3 (pg/ml)	1.178± 0.011	0.528± 0.003*
	FT4 (ng/dL)	0.529±0.003	0.513±0.003
Liver function Profile	**AST** (U/L)	121.873 ± 3.624	253.707±10.549*
	ALT (U/L)	24.136 ± 1.278	229.841 ± 9.205*
	Total protein (g/dL)	6.198 ± 0.097	3.755 ± 0.061*
	Albumin (g/dL)	4.156 ± 0.041	2.013 ± 0.021*
	Globulin (g/dL)	2.042 ± 0.022	1.742± 0.015*
Cytokines Profile	**Serum TNF–α** (pg/ml)	5.177 ± 0.049	11.043 ± 0.087*
	Serum IL-1ß (pg/ml)	3.802 ± 0.037	6.291 ± 0.061*
	Serum IL-12 (pg/ml)	11.059±0.018	24.085±0.074*
	Serum IL-4 (pg/ml)	55.49±0.117	197.51±1.429*
Tissues liver	GSH(mg/g tissue)	27.562±0.184	14.971±0.095*
	Gpx(µmol/min/g tissue)	129.527±1.328	78.524±0.806*
	TBARS(nmol/100 mg tissue)	1.826±0.017	4.413±0.039*

- n = number of rats. **- (*) refer to significance (P < 0.001).**

Liver glutathione (GSH) activity was decreased in Acetamiprid rats. On detecting liver glutathione peroxidase from data tabulated in Table (1). It is denoted that normal control animals had constant levels of liver glutathione peroxides during the investigation . On the other hand, in the current study, liver TBARS showed significant (P< 0.001) increase in the mean values of Acetamiprid rats.

In the current investigation (Table 2), amelioration effect occurred in the serum LDH, AST and ALT activities of rats group that were treated with ginseng, green tea, cinnamon and mixture for 15 and 30 days depending on the time of treatment (2 and 4 weeks).

Table (2): Amelioration effects of ginseng, green tea, cinnamon and their mixture on liver function profile in male albino rats (Mean ± SE).

Treatment / Interval	Control	Acetamiprid recover	Acetamiprid + ginseng	Acetamiprid + green tea	Acetamiprid + cinnamon	Acetamiprid + Mixture
	AST (U/L)					
15 days n = 5	122.147 ±3.631$^{A}_{a}$	210.439 ±8.775$^{B}_{a}$	180.522 ±7.003$^{C}_{a}$	184.439 ±7.095$^{C}_{a}$	186.106 ±7.112$^{C}_{a}$	169.524 ±5.659$^{D}_{a}$
30 days n = 5	124.023 ±3.639$^{A}_{a}$	179.327 ±6.981$^{B}_{b}$	159.701 ±5.697$^{C}_{b}$	170.559 ±6.311$^{D}_{b}$	171.201 ±6.358$^{D}_{b}$	140.327 ±4.469$^{E}_{b}$
	ALT (U/L)					
15 days n = 5	24.135 ±1.274$^{A}_{a}$	177.299 ±7.101$^{B}_{a}$	110.364 ±5.892$^{C}_{a}$	132.247 ±6.225$^{D}_{a}$	136.491 ±6.297$^{D}_{a}$	85.627 ±4.064$^{E}_{a}$
30 days n = 5	24.139 ±1.277$^{A}_{a}$	105.556 ±5.068$^{B}_{b}$	62.473 ±3.192$^{B}_{b}$	88.737 ±4.399$^{B}_{b}$	90.448 ±4.416$^{B}_{b}$	38.816 ±2.352$^{E}_{b}$
	Total protein (g/dL)					
15 days n = 5	6.203 ±0.095$^{A}_{a}$	4.054 ±0.072$^{B}_{a}$	4.927 ±0.082$^{C}_{a}$	4.576 ±0.081$^{D}_{a}$	4.569 ±0.081$^{D}_{a}$	5.137 ±0.086$^{E}_{a}$
30 days n = 5	6.197 ±0.095$^{A}_{a}$	4.568 ±0.079$^{B}_{b}$	5.498 ±0.089$^{C}_{b}$	5.105 ±0.085$^{D}_{b}$	5.093 ±0.084$^{D}_{b}$	6.199 ±0.092$^{A}_{b}$
	Albumin (g/dL)					
15 days n = 5	4.153 ±0.043$^{A}_{a}$	2.259 ±0.028$^{B}_{a}$	2.883 ±0.034$^{C}_{a}$	2.569 ±0.031$^{D}_{a}$	2.562 ±0.031$^{D}_{a}$	3.104 ±0.036$^{E}_{a}$
30 days n = 5	4.157 ±0.044$^{A}_{a}$	2.721 ±0.032$^{B}_{b}$	3.631 ±0.028$^{C}_{b}$	3.104 ±0.035$^{D}_{b}$	3.089 ±0.034$^{D}_{b}$	4.146 ±0.041$^{A}_{b}$
	Globulin (g/dL)					
15 days n = 5	2.050 ±0.022$^{A}_{a}$	1.795 ±0.016$^{B}_{a}$	2.044 ±0.020$^{A}_{a}$	2.007 ±0.016$^{A}_{a}$	2.007 ±0.017$^{A}_{a}$	2.033 ±0.019$^{A}_{a}$
30 days n = 5	2.040 ±0.021$^{A}_{a}$	1.847 ±0.017$^{B}_{b}$	1.867 ±0.016$^{B}_{b}$	2.001 ±0.017$^{A}_{a}$	2.004 ±0.017$^{A}_{a}$	2.053 ±0.017$^{A}_{a}$

A, B, C, D, E Means with a common superscript within a row are significantly different at (P<0.05).
a, b Means with a common subscript within a column are significantly different at (P<0.05).

Table (3): Amelioration effects of ginseng, green tea, cinnamon and their mixture on lipid profile in male albino rats (Mean ± SE).

Treatment / Interval	Control	Acetamiprid recover	Acetamiprid + ginseng	Acetamiprid + green tea	Acetamiprid + cinnamon	Acetamiprid + Mixture
	Serum cholesterol (mg/dL)					
15 days n = 5	55.493 ±0.842$^{A}_{a}$	98.548 ±1.351$^{B}_{a}$	80.771 ±1.103$^{C}_{a}$	85.404 ±1.278$^{D}_{a}$	85.892 ±1.288$^{D}_{a}$	74.292 ±1.351$^{E}_{a}$
30 days n = 5	54.891 ±0.837$^{A}_{a}$	81.692 ±1.211$^{B}_{b}$	68.545 ±1.008$^{C}_{b}$	77.912 ±1.078$^{D}_{b}$	78.331 ±1.081$^{D}_{b}$	59.428 ±0.942$^{E}_{b}$
	Serum triglycerides (mg/dL)					
15 days n = 5	64.559 ±0.937$^{A}_{a}$	121.652 ±1.62$^{B}_{a}$	95.319 ±1.318$^{C}_{a}$	100.897 ±1.524$^{D}_{a}$	102.004 ±1.533$^{D}_{a}$	88.727 ±1.196$^{E}_{a}$
30 days n = 5	64.692 ±0.952$^{A}_{a}$	100.709±1.511$^{B}_{b}$	82.116 ±1.089$^{C}_{b}$	85.921 ±1.094$^{D}_{b}$	86.117 ±1.102$^{D}_{b}$	70.556 ±0.971$^{E}_{b}$
	Serum HDL-Ch (mg/dL)					
15 days n = 5	14.601 ±0.022$^{A}_{a}$	20.329 ±0.051$^{B}_{a}$	17.117 ±0.039$^{C}_{a}$	18.652 ±0.043$^{D}_{a}$	18.713 ±0.045$^{D}_{a}$	16.218 ±0.034$^{E}_{a}$
30 days n = 5	14.578 ±0.021$^{A}_{a}$	18.776±0.046$^{B}_{b}$	16.328 ±0.046$^{C}_{b}$	17.009 ±0.046$^{D}_{b}$	17.106 ±0.046$^{D}_{b}$	14.588 ±0.029$^{E}_{b}$
	Serum LDL-Ch (mg/dL)					
15 days n = 5	27.980 ±0.033$^{A}_{a}$	53.889 ±0.091$^{B}_{a}$	44.590 ±0.077$^{C}_{a}$	46.573 ±0.080$^{D}_{a}$	46.778 ±0.081$^{D}_{a}$	40.329 ±0.066$^{E}_{a}$
30 days n = 5	27.365 ±0.033$^{A}_{a}$	42.77 ±0.07$^{B}_{b}$	35.794 ±0.054$^{C}_{b}$	43.719 ±0.075$^{D}_{b}$	44.002 ±0.077$^{D}_{b}$	30.729 ±0.042$^{E}_{b}$
	FT3 (pg/ml)					
15 days n = 5	1.178 ±0.011$^{A}_{a}$	0.597 ±0.004$^{B}_{a}$	0.742 ±0.008$^{C}_{a}$	0.663 ±0.006$^{D}_{a}$	0.657 ±0.006$^{D}_{a}$	0.859 ±0.009$^{E}_{a}$
30 days n = 5	1.184 ±0.012$^{A}_{a}$	0.639 ±0.005$^{B}_{b}$	0.957 ±0.010$^{C}_{b}$	0.801 ±0.007$^{D}_{b}$	0.789 ±0.007$^{D}_{b}$	1.179 ±0.005$^{E}_{b}$
	FT4 (ng/dL)					
15 days n = 5	0.529 ±0.003	0.513 ±0.003	0.519 ±0.003	0.524 ±0.003	0.517 ±0.003	0.525 ±0.003
30 days n = 5	0.522 ±0.003	0.526 ±0.003	0.524 ±0.003	0.518 ±0.003	0.513 ±0.003	0.520 ±0.003

A, B, C, D, E Means with a common superscript within a row are significantly different at (P<0.05).
a, b Means with a common subscript within a column are significantly different at (P<0.05).

In this study, the reciprocal relationship between the concentrations of thyroid hormones (FT3 and FT4) in serum induced by Acetamiprid was evident (Table 3). The maximum hepatoprotective effect was observed in the rat groups that were treated with both antioxidants (ginseng + green tea + cinnamon) depending on the time of supplementation

Table (4): Amelioration effects of ginseng, green tea, cinnamon and their mixture on cytokines profile in male albino rats (Mean ± SE).

Treatment / Interval	Control	Acetamiprid recover	Acetamiprid + ginseng	Acetamiprid + green tea	Acetamiprid + cinnamon	Acetamiprid + Mixture
	IL-12					
15 days n = 5	11.061 ±0.018 $^{A}_{a}$	21.721 ±0.068 $^{B}_{a}$	15.568 ±0.032 $^{C}_{a}$	17.013 ±0.048 $^{D}_{a}$	16.978 ±0.046 $^{D}_{a}$	14.005 ±0.029 $^{E}_{a}$
30 days n = 5	11.064 ±0.019 $^{A}_{a}$	18.903 ±0.061 $^{B}_{b}$	12.782 ±0.026 $^{C}_{b}$	15.231 ±0.037 $^{D}_{b}$	15.404 ±0.039 $^{D}_{b}$	11.127 ±0.021 $^{E}_{b}$
	IL-4					
15 days n = 5	56.101 ±0.122 $^{A}_{a}$	185.67 ±1.179 $^{B}_{a}$	129.72 ±0.985 $^{C}_{a}$	151.11 ±1.007 $^{D}_{a}$	150.94 ±1.009 $^{D}_{a}$	112.86 ±0.819 $^{E}_{a}$
30 days n = 5	56.087 ±0.121 $^{A}_{a}$	152.89 ±1.016 $^{B}_{b}$	97.36 ±0.761 $^{C}_{b}$	117.06 ±0.974 $^{D}_{b}$	115.77 ±0.981 $^{D}_{b}$	73.41 ±0.486 $^{E}_{b}$
	IL-1ß					
15 days n = 5	3.802 ±0.037$^{A}_{a}$	6.003 ±0.061$^{B}_{a}$	5.118 ±0.048$^{C}_{a}$	5.122 ±0.049$^{C}_{a}$	4.747 ±0.043$^{D}_{a}$	4.041 ±0.038$^{A}_{a}$
30 days n = 5	3.802 ±0.037$^{A}_{a}$	5.612 ±0.054$^{B}_{b}$	4.006 ±0.041$^{C}_{b}$	4.321 ±0.044$^{D}_{b}$	3.924 ±0.039$^{C}_{b}$	3.809 ±0.038$^{A}_{a}$
	TNF-α					
15 days n = 5	5.175 ±0.048$^{A}_{a}$	10.129 ±0.078$^{B}_{a}$	8.282 ±0.058$^{C}_{a}$	9.005 ±0.067$^{D}_{a}$	7.537 ±0.056$^{E}_{a}$	5.175 ±0.048$^{A}_{a}$
30 days n = 5	5.178 ±0.049$^{A}_{a}$	9.096 ±0.071$^{B}_{b}$	6.778 ±0.053$^{C}_{b}$	8.174 ±0.061$^{D}_{b}$	5.202 ±0.048$^{A}_{b}$	5.178 ±0.049$^{A}_{a}$

A, B, C, D, E Means with a common superscript within a row are significantly different at (P<0.05).
a, b Means with a common subscript within a column are significantly different at (P<0.05).

Supplementation of ginseng or green tea or cinnamon or mixture significantly inhibited ($P < 0.05$) the release of serum inflammatory markers and neutrophil infiltration as well as attenuated TNF- α, IL-1β IL- 4 and IL- 12 levels (Table 4).

In contrast, administration of ginseng or green tea or cinnamon or mixture ameliorated the depletion of intracellular GSH levels, modulated the production of MDA and significantly inhibited and reduced the elevated activities of MPO compared with ACP group (Table 5).

Table (5): Amelioration effects of ginseng, green tea , cinnamon and their mixture on Tissues liver profile in male albino rats (Mean ± SE).

Treatmen / Interval	Control	Acetamiprid recover	Acetamiprid + ginseng	Acetamiprid + green tea	Acetamiprid + cinnamon	Acetamiprid + Mixture
	Tissues liver GSH (mg/g tissue) content					
15 days n = 5	27.702 ±0.187 $^{A}_{a}$	16.429 ±0.101 $^{B}_{a}$	19.281 ±0.132 $^{C}_{a}$	17.839 ±0.118 $^{D}_{a}$	17.928 ±0.117 $^{D}_{a}$	22.026 ±0.142 $^{E}_{a}$
30 days n = 5	27.593 ±0.185 $^{A}_{a}$	18.874 ±0.124 $^{B}_{b}$	22.929 ±0.129 $^{C}_{b}$	19.984 ±0.121 $^{D}_{b}$	19.979 ±0.121 $^{D}_{b}$	25.114 ±0.163 $^{E}_{b}$
	Tissues liver Gpx (µmol/min/g tissue) activity					
15 days n = 5	129.498 ±1.325 $^{A}_{a}$	85.638 ±0.886 $^{B}_{a}$	95.494 ±0.923 $^{C}_{a}$	90.021 ±0.904 $^{D}_{a}$	89.997 ±0.898 $^{D}_{a}$	100.546 ±1.117 $^{E}_{a}$
30 days n = 5	129.504 ±1.327 $^{A}_{a}$	96.221 ±0.947 $^{B}_{b}$	107.402 ±1.189 $^{C}_{b}$	100.558 ±1.124 $^{D}_{b}$	100.003 ±1.109 $^{D}_{b}$	117.253 ±1.265 $^{E}_{b}$
	Tissues liver TBARS (nmol/100 mg tissue) level					
15 days n = 5	1.828 ±0.018 $^{A}_{a}$	4.006 ±0.032 $^{B}_{a}$	3.018 ±0.026 $^{C}_{a}$	3.729 ±0.028 $^{D}_{a}$	3.736 ±0.028 $^{D}_{a}$	2.502 ±0.022 $^{E}_{a}$
30 days n = 5	1.831 ±0.019 $^{A}_{a}$	3.327 ±0.027 $^{B}_{b}$	2.623 ±0.024 $^{C}_{b}$	3.009 ±0.025 $^{D}_{b}$	3.018 ±0.026 $^{D}_{b}$	1.838 ±0.019 $^{A}_{b}$

A, B, C, D, E Means with a common superscript within a row are significantly different at (P<0.05).
a, b Means with a common subscript within a column are significantly different at (P<0.05).

DISCUSSION

Acetamiprid is a source of toxicities in humans. Its creation takes place during the thermal processing of foods that contain ACP. Where the majority of ACP was conjugated with glutathione while a minimal amount was activated via glycidamide. Both ACP and glycidamide can form covalent adducts with DNA and hemoglobin, as well as induced gene mutation, chromosomal aberration and malignant neoplasm in rodents. Furthermore, ACP and its bio-transformed metabolite, glycidamide, are hazardous to different organs such as the liver.

In the present work, the activities of LDH, ALT and AST in ACP group were significantly elevated, suggesting the aggressiveness of ACP with time and confirming the advanced permeability of the hepatic membrane after 4 weeks, due to oxidative stress. Up regulation of pro-inflammatory cytokine expression can also be associated with the generation of free radicals in macrophages at the inflammation stage. The plasma levels of the pro-inflammatory cytokines TNF-α, IL-1β, IL-4 and IL-12 4 were significantly increased in ACP group, revealing hepatic injury; as well as over production of ROS. In addition, significant increase in products of lipid peroxidation, was also observed in ACP group suggesting a relationship between ACP and oxidative stress, and is an indicator of hepatic tissue damage.

GSH is a major intracellular antioxidant as well as an important component in metabolism of many xenobiotic. Recently, it was reported that AA is an oxidative stress inducer. It is efficiently conjugated with GSH to form glutathione-AA adduct that can create cellular oxidative stress. GSH depletion leads to increased lipid Peroxidation and necrosis of hepatocytes

Also the activity of glutathione-s-transferase (GST) which is a multifunctional enzyme and plays a key role in cellular detoxification did not affected by the treatments. In addition, the lipid profile was slight increase in ACP group these findings are apparently agreement with [34] who revealed that ACP could stimulate oxidative toxicity and reproductive toxicity in male rats administrated orally 100 mg/Kg body weight (1/10 oral LD50) daily for 30 days. It is obvious the different of the quantity of dose that rat received. Also if we take in consideration the different in tolerance between the species [6] found that ACP administered 40 mg/kg body weight (1/5th of LD50 value) per day for 28 days induces prominent toxic symptoms with hematological and biochemical effect in mice but 10 mg/kg body weight (1/20 of LD50 value) proved to be non-toxic for the experimental animals. More addition [28] reported that the ACP were administered orally to rats at (25, 100 and 200 mg/kg of body weight) for a period of 28 days, the high dose made extensive neurotoxic effects but low dose did not induced such effects. So, It seems that the ACP made the adverse effect according to the quantity of dose given and the kind of species.

Significant increase in plasma AST activity in Acetamiprid treated rats suggested an increased respiratory burst and mitochondrial involvement, as AST was chiefly a mitochondrial enzyme. Mitochondrion plays an important role in maintaining hepatocyte integrity and function, which might be hampered due to excessive physiologic stress [35]. Activity of AST was high in acute and chronic liver injury [36]. Elevation in the AST could be associated with cell necrosis of many tissues. Pathology involving the skeletal or cardiac muscle and/or the hepatic parenchyma, allowed the leakage of large amount of this enzyme into blood [37]. The elevation in AST produced by Acetamiprid is an indication of tissue damage. Also [28] reported increased AST activity in female Wistar rat after Acetamiprid administration which was in well agreements with the present finding. Alanine amino transferase was a key cytoplasmic enzyme present in liver and other cells. It was particularly useful in measuring hepatic necrosis, especially in small animals [38]. Alanine amino transferase was considered as a marker of hepato cellular damage and in general ALT was considered a more sensitive indicator of liver cell injury than AST [39]. Though AST and ALT were not known to have any function in the plasma, but their increase level in the blood indicate cellular damage and increased membrane permeability [40] and their altered metabolism [41]. Since ALT was one of the specific assayable liver enzymes, its elevated level in the study may indicate hepatic damage caused by oral administration of Acetamiprid. Although it was difficult to point the damage to any particular organ by Acetamiprid, but increased levels of aminotransferases in rats may be attributed to liver damage, as a primary organ of biotransformation of Acetamiprid. The Acetamiprid caused decreased in globulin. Decreased levels of globulin indicate that the immune competence of the animals will be easily compromised. As a matter of fact, lymphopaenia accompanied by low globulin level, may lead to immunosuppression. In case of decreased globulin level, diseases characterized by deficiency of immunoglobulin, such as agammaglobulinaemia selective IgM, IgA and IgG deficiencies and transient hypogammaglobulinaemia, may lead to low level globulin [32]. Acetamiprid might possess immunosuppressive properties as well as causing inflammatory effects.

Mondal [42] performed oral administration of Acetamiprid to female wister rats; [43] found a significant shift in the blood biochemical parameters in Acetamiprid oral administration in goat. [44] confirmed significant increase in activity of serum Alanine transaminase (ALT) of male mice in Acetamiprid toxicity. Bhardwaj et al. [45] reported elevation in GPT in imidacloprid toxicity in female rats. The present findings of increase in the value of AST was in agreement with the findings of [45] in female rats following administration of Imidacloprid and [44] in male mice following administrations of Acetamiprid. Increase in Alkaline phosphatase value was also reported by other workers such as Acetamiprid toxicity in female rats [2], Acetamiprid in male mice [44], dichlorovos in male mice [46]. The increase in ALP usually occurred due to its increased synthesis due to damaged liver conditions [47]. Elevated plasma ALP might be due to acute hepatocellular damage and destruction of epithelial cells in gastrointestinal tracts [48].

It is interesting to observe that rats treated with ginseng had a significant improvement of AST and ALT enzymes, and had a significant suppression of TNF-α, IL-1β, IL-4 and IL-12; compared to recovery rats. The GSH content was significantly enhanced, The present study demonstrates the antioxidant activity of ginseng against ACP-induced hepatotoxicity. Ginseng extracts have been reported to show protective effects on hepatocytes in vitro and liver injury in various animal, and clinical models induced by a wide variety of hepatotoxins including hydrogen peroxide(H2O2) [49], alcohol [50], carbon tetrachloride(CCl4) [51], aflatoxin B1 [52] and fumonisins [53].

The mechanisms which provide ginseng's hepatoprotective effects are closely attributed to antioxidation properties. Ginseng enhanced the antioxidant defense mechanism and increased self-antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GSH), and heme oxygenase-1 in the aged-rat liver and hepatotoxins-induced liver damages in rats [54]. Ginseng treatments inhibited oxidative stress damage such as lipid peroxidation [55], malondialdehyde, thiobarbituric acid reactive substance, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) **.** The protective effects have been histologically and histochemically monitored. Recently, further molecular mechanism studies found that ginseng suppresses mitogen-activated protein kinase (MAPK) signals [49], nuclear factor-kappa B (NF-kB), and inducible nitric oxide synthase (iNOS) protein expression. Anti-Inflammation. Inflammatory effects of ginseng have been responsible for the liver protection. Ginseng suppressed the production of inflammatory cytokines (IL-1β, IFN- γ) and chemokines (MCP-1, MIP-2β, KC) in CCl4-treated mice [55]. Recently, ginseng was found to inhibit tumor necrosis factor alpha (TNF-α)-stimulated NF-kB activation and further suppressed the gene expression of iNOS and cyclooxygenase-2 (COX-2) in HepG-2 cells [56].

Supplementation of green tea significantly ameliorated liver function as indicated by AST and ALT levels and the production of pro inflammatory cytokines, as well as improved hepatic antioxidant defense capacity. Green tea increases the activity of liver antioxidative enzymes GSH-Px and GSSG-R, and the content of reduced glutathione (GSH), improves total

antioxidant status (TAS) and diminishes the level of lipid peroxidation products, measured as lipid hydroperoxides, 4-HNE and MDA. The increase in enzyme activities in the liver could be explained by the induction of rat hepatic drugmetabolising enzymes at the gene level following dietary administration of tea polyphenols [57]. Their rise with the intracellular increase in liver GSH, where it is synthesised, potentiates the antioxidative status of the liver after green tea ingestion. In addition, GSH is a major source of reducing power and normally exists intracellularly at high concentrations. It is maintained largely in the reduced form by glutathione reductase, acting in conjunction with NADPH. GSH has been reported to be involved in protein and DNA biosynthesis, maintenance of cell membrane integrity and regulation of enzyme activity [58].

The improvements recorded in all the previous parameters due to Supplementation of cinnamon explained by Cinnamomum possesses significant ant allergic, antiulcerogenic, antipyretic and anesthetic activities [59]. The bark yields an essential oil containing cinnamaldehyde and eugenol. Several biological activities, such as peripheral vasodilator, antitumor, antifungal, cytotoxic and antimutagenic, have been attributed to cinnamaldehyde [60].

Conclusion

The current investigation demonstrates that combination of gensing, green tea and cinnamon possesses a good potential to inhibit oxidative stress from liver and exhibit anti-inflammatory activity through synergistic properties GSH levels, thereby, reducing the liver injury induced by ACP. Further physiological, biochemical and histopathological investigations are needed to explore the possible use of different doses of these crude extracts and their constituents as potential natural preventive agents against the influences of Acetamiprid and may be against other toxicants and pathogenic factors.

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