sJournal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/1474
DOI URL: http://dx.doi.org/10.21474/IJAR01/1474
ISSN NO. 2320-5407

RESEARCH ARTICLE

HEPATOPROTECTIVE EFFECTS OF AGED GARLIC AND ETHANOLIC GINGER EXTRACTS IN MICE.

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Manuscript Info

Manuscript History

Received: 12 July 2016
Final Accepted: 19 August 2016
Published: September 2016

Key words:-
Acetaminophen, alanine aminotransferase garlic, ginger, glutathione

Abstract

Acetaminophen (APAP) is a widely used over-the-counter analgesic and antipyretic drug. .At large doses, it produces a fatal hepatic necrosis that accounts for acute liver failure. The present study investigates the potential protective effect of prior administration of ethanolic ginger and aged garlic extracts against acetaminophen-induced hepatic injury. Liver glutathione (GSH) and serum levels of alanine aminotransferase (ALT) and bilirubin were measured from groups of mice given APAP alone, orally as a suspension in distilled water in a dose of 500mg/kg, after prior pretreatment with oral 250 and 500µgof ginger extract/d in 1.1% alcohol and after prior intraperitoneal administration of aged garlic extract in dose of 100 and 200 mg/kg. The present study showed that significant improvement ($p< 0.05$) of both hepatic glutathione levels and serum levels of ALT and bilirubin in mice after prior administration of either ethanolic ginger extract or aged garlic extract each alone or in combination with each other compared with mice given APAP alone (in which hepatic GSH was significantly decreased and serum ALT and bilirubin were significantly increased, $p< 0.05$). The present work suggests that both ethanolic ginger extract and aged garlic possess hepatoprotective effects against acetaminophen-induced hepatic injury in mice.

Introduction:-

Acetaminophen (APAP) is an effective analgesic and antipyretic agent. It is safer compared with non-steroidal anti-inflammatory drugs if used as directed by the physician. However, if used in large doses, APAP may cause serious liver injury. That leads to acute liver failure (1-2). APAP hepatic injury has been explained by the formation of N-acetyl-p-benzoquinone imine (NAPQI) in the liver that causes oxidative stress by depletion of the antioxidant glutathione. Additionally, NAPQI binds to important mitochondrial proteins causing necrosis of liver cells and release of inflammatory cytokines (3-4).

Use of natural antioxidants in food nowadays is considered to be a promising alternative for synthetic antioxidants owing to its low cost, high compatibility with dietary intake and harmless effects. Garlic and ginger are health

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protecting foods that contain rich amount of beneficial antioxidants. Garlic has long been used for hypertension and diabetes (5-7). It is owed to its antioxidant/anti-inflammatory and neuroprotective activities. Aged garlic extracts (AGE), compared with fresh garlic preparations, has no odor, contains higher quantities of antioxidants and protects against cancer, many cardiovascular diseases, stroke, Alzheimer's disease and other aging disorders (8). To get higher quantities of antioxidants, AGE is prepared by extracting and aging fresh garlic at room temperature for about one and half years in 20 % ethanol. AGE contains flavonoids, allixin, selenium and water-soluble sulfur antioxidant substances with high bioavailability such as S-allylmercaptocysteine (SAMC) and S-allyl cysteine (SAC) (9-10).

Ginger is used in many foods and beverages. Ginger oil that comprises 1-3% of its weight contains a high percentage of hydrocarbons, mainly zingiberene, bisapolene, and zingiberol (11-13). It is used in traditional medicine since thousands of years in many countries like China and India for relieve of some clinical conditions e.g. nausea, headache, colds and rheumatic disorders (14). Additionally, is used in China for cold extremities and after blood loss to resuscitate the patient (15). Ginger also has a beneficial in treatment of gastric ulcers (16-17). Recent studies in literature revealed that ginger has an anti-oxidative stress activity and neuroprotective effects that may be due to an influence on inhibitory and excitatory neurotransmitters, and calcium channel inhibition (18-21).A study by Vishwakarma and his colleagues suggested that the benzene fraction of a petroleum ether extract of dried rhizomes of ginger has anticonvulsant, anxiolytic, antiemetic,sedative and analgesic activities(22-23).

The present study aims at investigating the potential beneficial effects of both AGE and ethanolic ginger extracts in protection against APAP-induced hepatic injury in mice by measuring the hepatic content of the antioxidant glutathione in addition to serum levels of the liver enzyme Alanine aminotransferase (ALT) and bilirubin that increase between 24 and 72 hours following exposure to an overdose of APAP.

Materials and Methods:-

Chemicals and kits:-

APAP tablet (Panadol®) suspended in freshly prepared distilled water (DW). Based on literature (24-25), oral median lethal dose (LD50) of APAP in mice equals 400 - 900 mg/kg thus 500mg/kgAPAP was used to induce hepatic injury in mice. .Hepatic glutathione (GSH) was measured by using GSH Assay Kit (Cayman Chemical Company, United States).

Animals:-

Male mice (5 weeks) weighting 20-30 grams were acclimatized for one week in at room temperature (23±3ºc) on a 12-hours light-dark cycle and were fed *ad libitum.*

Preparation of ethanolic extract of ginger:-

According to the method described by Fuhrman and his colleagues, a stock solution was prepared by dissolving 200 mg of ginger extractin 44 mL of ethanol and then adjusted to 200 mL with DW. A concentration of 50 mg/L of ginger extract in 1.1% alcohol and water was prepared by dilution of 25 mL of the ginger extract stock solution into 500 mL of DW (26).

Preparation of aged garlic extracts (AGE):-

Following method described Fatemeh and her colleagues, garlic bulbs were peeled and minced in an aqueous-alcoholic solution and maintained for 18 months in anaerobic conditions. The aged garlic was then crushed using mortar and pestle and homogenized in DW. Using Whatman paper No. 1, the prepared homogenized aged garlic was filtered and then centrifuged to get the cleared supernatant. A stock solution400mggarlic /mlDW was prepared (27).

Experimental protocol:-

After acclimatization for one week, micewere randomly divided into 7groups 10 animals each. Control group was given 0.5 mlDW orally by intragastric tube. APAP was given to the 2nd group orally, as a 0.5 ml suspension in DW in a dose of 500 mg/kgonce. To the 3rdand 4thgroups 250 µg and 500 µg, respectively, of ginger extract in 1.1% alcohol and water and APAP in a dose of 500 mg/kg once. The 5th and6th mice groups were given intraperitoneally 100 mg/kg and 200mg/kg, respectively of AGEand APAP in a dose of 500 mg/kg. A combination of 500 µgginger extract in 1.1% alcohol and waterand 200mg/kgAGE in addition to APAP in a dose of 500 mg/kg was given to mice of the 7th group.

Measurement of Alanine aminotransferase levels:-
Fasting blood was obtained by decapitationand collected from each mouse into in EDTA-collecting tubes, and then centrifuged at 1,200 × g for 5 min at room temperature to obtain the serum sample, which was stored frozen at -20°C until analyzed. Alanine aminotransferase (ALT) was determined by method of Reitman and Frankel (28).

Measurement of total bilirubin levels:-
Total plasma bilirubin levels were measured following protocoldescribed byBortolussiet al. according to methodology of Doumasand his colleagues (29-30). Dilution of a stock solution of bilirubin (20 mg/dl) in BSA (4 g/dl)was prepared to perform standard curves. Absorbance values at 600 nm were obtained by using a multiplate reader (LD 400C Luminescence Detector, Italy).

Measurement of hepatic glutathione (GSH):-
Twenty-four hours following administration of APAP either alone or with ethanolic ginger or AGE, the liver was removed to determine hepatic hepatic glutathione (GSH) following methodology described by Werawatganonand his colleagues (31) using Cayman' GSH assay kit which utilizes a carefully optimized recycling method, using GSH reductase, for the quantification of GSH. The sulfhydryl group of GSH reacts with 5, 5′-ditrio-bis-(2-nitrobenzoic acid) (DTNB), or Ellman's reagent and produces a yellow colored 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB (between GSH and TNB) is reduced by GSH reductase to recycle the GSH and produce more TNB. The production of TNB is directly proportional to this recycling reaction which is in turn directly proportional to the concentration of GSH in the sample. The optical density of TNB is then measured at 405–414 nm using a microplate reader, which provides an accurate estimation of GSH in the sample.

Statistical analysis:-
Data of the present work are presented as mean ± SE. One-way analysis of variance(ANOVA) was used to test groups' differences (Statistical significance was considered at $p<0.05$) by using SPSS version 22.

Results:-

Alanine aminotransferase levels:-
The present work observed that serum ALT was significantly increased(237.6 ± 2.87 U/L vs51.25 ± 2.72U/L, $p< 0.05$) in the positive control group (given APAP) compared with the negative control group (given DW). Compared with positive control group, serum levels of ALT were lowered significantly in groups given ginger extracts in doses of 250 and 500µg with APAP (60.12 ± 2.41U/L and 55.2 ± 2.32U/L vs237.6 ± 2.87 U/L, $p< 0.05$, respectively). Similarly, ALT levels in sera of mice given AGE in doses of 100 and 200 mg/kg were significantly lower compared with levels in mice given APAP alone (56. 2 ± 1.88U/Land 53.1 ± 2.1 U/L vs237.6 ± 2.87 U/L, $p< 0.05$, respectively. Combination of 500µg ginger extract and 200 mg/kg AGE significantly improved ALT levels in mice given APAP (52.3 ± 2.2 U/Lvs237.6 ± 2.87 U/L, $p< 0.05$.

Animal group (n=10)	Alanine aminotransferase (ALT) (U/L)	Total bilirubin level (mg/dl)	Hepatic glutathione (GSH) (nmol/mg protein)
negative control group given DW	51.25 ± 2.72	0.45±0.03	12.25±0.2
Positive control group given APAP 500 mg/kg	237.6 ± 2.87*	0.88±0.1*	3.3±0.01*
250 µg ginger extract+ APAP 500 mg/kg	60.12 ± 2.41#	0.55±0.01#	14.25±0.1#
500µg ginger extract+ APAP 500 mg/kg	55.2 ± 2.32#	0.5±0.02#	13.72±0.11#
100 mg/kg AGE+ APAP 500 mg/kg	56. 2 ± 1.88#	0.54±0.03#	13.22±0.2#
200 mg/kg AGE+ APAP 500 mg/kg	53.1 ± 2.1#	0.48±0.01#	12.75±0.1#
500µg ginger extract+ 200 mg/kg AGE+ APAP 500 mg/kg	52.3 ± 2.2#	0.46±0.02#	12. 5±0.2#

*significant versus negative control group ($p<0.05$)
#Significant versus positive control group ($p<0.05$)

Total bilirubin levels:-
Total bilirubin level was significantly increased(0.88±0.1mg/dlvs0.45±0.03, p< 0.05) in APAP group compared with control group. In groups given ginger extracts in doses of 250 and 500µg with APAP, total bilirubin levels were significantly lower compared with APAP alone (0.55±0.01mg/dland 0.5±0.02mg/dlvs0.88±0.1mg/dl, p< 0.05, respectively). Total bilirubin levels in mice given AGE in doses of 100 and 200 mg/kg with APAP were also significantly lower compared with those in mice given APAP alone (0.54±0.03mg/dl and 0.48±0.01mg/dlvs0.88±0.1mg/dl, p< 0.05, respectively. Given together, 500µg ginger extract and 200 mg/kg AGE significantly decreased bilirubin levels in mice given APAP (0.46±0.02 mg/dl vs0.88±0.1mg/dl, p< 0.05)

Hepatic glutathione (GSH):-
Levels of hepatic GSH decreased significantly in mice challenged with APAP compared with control group (3.3±0.01nmol/mg protein vs12.25±0.2 nmol/mg protein, P < 0.05). In mice ginger extracts in doses of 250 and 500µg with APAP,GSH levels increased significantly compared with APAP group (14.25±0.1 nmol/mg protein and 13.72±0.1101nmol/mg protein vs3.3±0.01nmol/mg protein, p< 0.001, respectively).In groups given AGE 100 and 200 mg/kg + APAP, GSH levels were also significantly higher compared with that group given APAP alone (13.22±0.201nmol/mg protein and 12.75±0.1nmol/mg protein vs3.3±0.01nmol/mg protein, p< 0.05, respectively. Concomitant administration of 500µg ginger extract and 200 mg/kg AGE to mice given APAP significantly increased GSH levels compared with control group given APAP alone (12. 5±0.2 vs3.3±0.01nmol/mg protein, p< 0.05).

Discussion:-

Acetaminophen (Paracetamol) is a commonly used safe and effective analgesic and antipyretic agent when used in therapeutic doses. However, when used at higher doses or misused by population, it could cause potential life-threatening hepatotoxicity. Between 24 and 72 hours following overdose, signs of liver damage start to appear in the form of right upper quadrant abdominal pain and the hepatic transaminases ALT and AST rise to abnormal levels (32).

The present study observed that after 24 hours of oral administration of 500 mg APAP to mice, serum levels of the hepatic enzyme ALT and the total bilirubin levels increased significantly compared to control mice with significant lowering of the hepatic antioxidant GSH. This is in agreement with other previous studies that reported rise oftransaminases and lowered hepatic GSH following paracetamol overdose (31, 33)

Ginger is a commonly used herbal supplement with antibacterial, antioxidant, and antiinflammatory effects (34-35). The present study showed a dramatic hepatoprotective activity following oral administration of ethanolic ginger extract in addition to APAP as evidenced by the highly significant improvement of serum levels of ALT and bilirubin and hepatic levels of GSH. Our observation agrees with other previous studies in literature. Atta and his colleagues observed that oral administration of methanol extract of ginger improved degenerative histopathological changes induced by CCl4intoxication in liver (36).An *in vitro* study showed that zingerone, a ginger metabolite, inhibited lipid peroxidation in rat liver (37).Shanmugam et al. have been reported a protective effect for dietary ginger against oxidative damage in experimental diabetic rat tissues (38). Moreover, administration of ginger suppressed gene expression of the hepatic inflammation markers, TNFα, and IL-6 and decreased NF-κB activity (39).

On the other hand, aged garlic, contrary to raw garlic, has been shown to have numerous beneficial biological effects antioxidant (40), anti-cancer (41) and immune stimulating effects of raw garlic (42). The present work showed that AGE exhibited a hepatoprotective effect against APAP-induced hepatic injury. Its concomitant intraperitoneal administration, in doses of 100 and 200 mg/kg, improved serum levels of ALT and billirubin and hepatic glutathione content. Our observation is in line withJung et al. who reported that aged black garlic (ABG) exerts hepatoprotective effects against hepatic injury induced by carbon tetrachloride CCl4-and D-galactosamine, and high fat diet -induced hepatic steatosis (43). Similarly, Kim and his colleagues concluded that ABG has strong antioxidative properties and may protect against chronic alcohol-induced liver damage (44).Another study by Shaarawy et al. showed that garlic and silymarinhave a synergistic hepatoprotectiveeffct against N-nitrosodiethylamine (NDEA) and CCl(4)-induced hepatotoxicity in male albino rats (45).Obioha et al. showed also that high dose of onion and moderate dose of garlic extracts ameliorated cadmium (Cd)-induced oxidative damage in rat liver by reduction of lipid peroxidation (46).In another model, Pal et al. reported that garlic protects against isoniazide and rifampin-induced hepatotoxicity (47).

In conclusion, the present study revealed that Co-administration of ginger and aged garlic extract could prevents against paracetamol-induce hepatic injury

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