

Full Length Research Paper

## Protective and therapeutic effects of *Chamomilla Recutita* extract on subacute ethanol intoxication in white albino rats

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Medicinal plants may provide an effective remedy for the enormous health burden posed by alcohol abuse. The aim of this study was to investigate putative protective and therapeutic effects of an aqueous extract of *Chamomilla recutita* (CHR) on alcohol-induced hepato-nephrotoxicity and pancytopenia in rats. Rats fed only alcohol developed hepato-nephrotoxicity as indicated by significant increases in the levels of all hepatic enzyme markers [alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GTT)], significantly decreased levels of total protein and increased levels of serum creatinine and urea. Ethanol administered rats also developed pancytopenia with decreased levels of total red blood cells (RBC's), white blood cells (WBC's) and platelets and macrocytic, hypochromic anemia. Oral administration of CHR extract for 28 days to normal rats confirmed its safe use with all the above biochemical and hematological parameters remaining within their normal levels. Pre-treatment or post-treatment with CHR to ethanol administered rats ameliorated the deleterious effects of ethanol on all biochemical and hematological parameters. CHR effect was more potent in pretreated- than in post-treated group. The results also show that a diminution of oxidative stress is one mechanism by which CHR extract exerts potent protective and mild therapeutic effects against alcohol-induced hepato-nephrotoxicity and pancytopenia.

**Key words:** *Chamomilla*, alcohol, liver, kidney, hematology, protection, therapy.

### INTRODUCTION

Well over two billion people worldwide consume alcoholic drinks, which may have immediate and long term consequences on their health and social lives. Alcohol-associated social problems include, but are not limited to, dependence, depression, domestic violence, family disruption and loss of work productivity. The global disease burden related to alcohol consumption, both in terms of morbidity and mortality, is considerable. In industrialized countries, heavy intake of alcohol is a leading cause of preventable mortality and morbidity, second only to

cigarette smoking (WHO Global Report on Alcohol, 2004). Long-term use of alcohol in excessive quantities is capable of damaging practically every organ system in the body with the most important effects, from a clinical point of view, relating to diseases of the circulatory, nervous and hepato-gastrointestinal systems (Testino, 2008). High levels of alcohol consumption are associated with an increased risk of physical trauma, cardiovascular disease, malabsorption, alcoholic liver disease, chronic pancreatitis, nephropathies, pancytopenia and cancers

(WHO Global Report on Alcohol, 2004). Damage to the central and peripheral nervous systems may also result from sustained alcohol consumption (Watson and Little, 2004). Ethanol is now recognized as the most prevalent known cause of abnormal human development (Testino, 2008). The adverse effects of ethanol are exerted directly or indirectly, through derangements in metabolic, hormonal and nutritional mechanisms (Amanvermez et al., 2005).

Numerous studies at national and international levels show that adverse effects of alcohol consumption on the gastrointestinal system range from increased intestinal transit time and gastropathies (characterized by classical early morning nausea and diarrhea), through significant malabsorption and alcoholic liver disease (fatty liver, alcoholic hepatitis, and cirrhosis) to chronic pancreatitis and cancers (Testino, 2008). Protein deficiency and enzyme activity impairment with decreased serum albumin concentration and changes in enzyme activities have been associated with alcoholic liver disease (Ozaras et al., 2003). Alcohol is the leading cause of liver cancer accounting for 32 to 45% of hepatic cancers in the western world and close to half a million people in the United States develop alcohol related liver cancer annually (Voigt, 2005).

The kidney is central to total body homeostasis, regulating extracellular water and electrolytes as well as acid base balance, among other critical functions. Renal damage may occur as a result of acute intoxication or chronic alcoholism (Vamvakas et al., 1998). As many as 65% of chronic alcoholics have IgA nephropathy at autopsy (Gonzalez-Quintela et al., 2008). The mechanism by which alcohol induces renal damage is uncertain. Free radical-induced lipid peroxidation (Nordman et al., 1992) has been put forward as playing a role.

Alcohol has widespread direct and indirect effects on the hematologic system which may mimic and or obscure other disorders. Leukocyte, erythrocyte and thrombocyte production and functions are affected directly by alcohol-induced toxicity to the bone. Liver damage secondary to alcohol abuse indirectly impacts red blood cells and the hemostatic mechanisms (Heemans, 1998). Since normal function of the hematopoietic system is dependent on the coordinated interplay of many other systems, it is not surprising that ethanol effects on the blood may be multiple, complex and highly variable. Alcohol is known to cause vacuolization in bone marrow cells and to suppress hematopoiesis (Yeung et al., 1988). However, this effect has not always been seen in the peripheral blood picture of alcoholics. Observed changes in their peripheral leukocytes, erythrocytes and other variables related to erythrocytes like packed cell volume (PCV) and hemoglobin (Hb), are contradictory and range from anemia and leucopenia to erythrocytosis and leucocytosis (Ballard, 1980). It has been suggested that alcohol may impair erythrocyte membrane fluidity and lipid composition (Beauge et al., 1987). Furthermore, it may cause an elevation of mean corpuscular volume (MCV) due, possibly, to a decrease in

vitamin folic acid concentration (Herbert et al., 1963).

Despite tremendous progress in the field, development of suitable medications for the treatment of ethanol toxicity remains a challenging goal for alcohol research. In the field of ethnopharmacology, there is an ongoing search for medicinal plants that may have protective effects against ethanol-induced tissue damage. Plant derived drugs are known to play a vital role in the management of toxicity and diseases. Focus on plant research has increased all over the world and a large body of evidence has been collected to show the immense potential of medicinal plants used in various traditional systems (Dahanukar et al., 2000). Such studies have led to the isolation of chemical substances with therapeutic properties and many of the isolates have found use as modern drugs while others have served as substrates for drug syntheses. Unfortunately, a greater proportion of plants known traditionally to possess medicinal properties and used in herbal medicine have not been subjected to scientific evaluation. Such evaluation is necessary not only because of the need to discover new drugs but also to estimate their toxicity risks. Besides, it is important that traditional claims of therapeutic properties of plants are scientifically confirmed even if the active principles are not ultimately discovered (Aliyu, 1994).

*Chamomilla recutita* (L.) (Chamomile) is one of the most popular and well documented herbal medicines in the Arab world. The flower-heads are used both systemically and locally to alleviate or even cure a vast list of disorders, particularly those related to inflammatory conditions (Mann and Staba, 1986). Although used in different pharmaceutical preparations, chamomile is most commonly taken as an infusion for sedative and anxiolytic purposes, as a digestive aid to treat gastrointestinal disturbances, especially in babies and small children (Madisch et al., 2001; Mahady et al., 1999), and externally for wetting cotton pads used for topical treatment of skin cuts, wounds and ulcers (Mills and Bone, 2000). Of particular relevance to this study is the recent finding of a gastroprotective effect of *C. recutita* extract against ethanol-induced gastric ulcers in rats (Al-Hashem, 2010). The aim of the current work therefore, was first to establish any protective and/or therapeutic effects of aqueous extract of *C. recutita* on liver and kidney toxicity and on hematological parameters in ethanol intoxicated white albino rats and then to investigate its mechanism of action.

## MATERIALS AND METHODS

### Aqueous extract preparation

*C. recutita* flowers were purchased from the local market in Abha, south western Saudi Arabia. The plant was identified by staff of the Department of Biology at King Khalid University. The flowers were washed with distilled water and then dried. The dried flowers were ground to a powder and extracted by maceration in distilled water (200 g/1500 mL, w/v) for two days at 37°C. The extract was filtered and excess water evaporated under reduced pressure in a rotary evaporator. The dry extract amounted to about 18 g of solid residue.

The extract was dissolved in distilled water to a final stock concentration of 10 g/ml for further use.

### Animals and experimental protocol

Male albino rats, each weighing about 200 g, were obtained from the Experimental Animal Unit, College of Medicine, King Khalid University, Saudi Arabia. They were maintained in a standard animal room, in well ventilated cages with raised floors of wide wire mesh to prevent them from feeding on their feces. They were kept at an ambient temperature of  $22 \pm 3^\circ\text{C}$  in 12 h light-dark cycles. The rats were fed a balanced diet and given free access to water. All animals were allowed to acclimatize for one week before ethanol and CHR extract treatments. The design of the experiments was approved by the Physiology Department Research Committee of King Khalid University. All procedures were performed in sterile conditions and conducted in accordance with internationally accepted principles of laboratory animal use and care as found in the United States guidelines (United States National Institute for Health publication no. 85: 923, revised in 1985).

Five groups of animals, each of six rats, were used. All treatments were administered orally to animals once daily for a period of 28 days using cavaige needles and stainless steel cannulae. Group 1 (Control) animals received isotonic saline solution (ISS, 1ml/ rat). Group 2 (ethanol treated rats) were given 1 ml of 70 % ethanol. Group 3 (CHR extract treated) rats received 1 ml of *C. recutita* aqueous extract at a dose of 1.0 g/kg body weight. Group 4 (Pre-treated) rats were given 1 ml of aqueous CR extract at a dose of 1.0 g/kg b.wt. for the first 14 days followed by daily administration of 1 ml of 70% ethanol for the remaining 14 days of the study period. Group 5 (post-treated) rats were first given 1 ml of 70% ethanol for the 14 days followed by daily administration of 1 ml of aqueous CR extract at a dose of 1.0 g/kg b.wt. for another 14 days.

### Hematological and biochemical estimations

Prior to termination of treatment on day 28, the rats were fasted overnight but distilled water was made available ad libitum. Blood samples were collected from rat tails under ether anesthesia. One fraction of blood was collected into EDTA-coated sample tubes for full blood count (FBC). The other fraction, collected into plain tubes, was centrifuged at 3000 rpm for 10 min to obtain serum for biochemical analyses. Hematological studies, including total RBC count, total WBC count, Hb, HCT, MCV, MCH, MCHC, platelets count and RBC Distribution Width (RDW-SD), were done using an Automated Hematology System (Sysmex Hematology-Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Kobe, Japan). The activities of aspartate amino transferase (AST, E.C. 2.6.1.1), alanine amino transferase (ALT, E.C. 2.6.1.2), alkaline phosphatase (ALP, E.C. 3.1.3.1) and gamma-glutamyl transferase (GGT, E.C. 2.3.2.2) in serum were measured with commercially available kits (Boehringer Mannheim, Mannheim, Germany). Serum urea was estimated using the diagnostic kit based on the method of Fawcett and Scott (1960). Serum creatinine was estimated using the diagnostic kit based on the method of Teitz (Burtiss and Ashwood, 1996). Total protein content was estimated by the method of Lowry et al. (1951).

### Preparation of liver and kidney homogenates

Livers and kidneys were quickly removed, washed in ice-cold, isotonic saline and blotted individually on ash-free filter paper. Some liver tissues were then homogenized separately in 0.1 M Tris-HCl buffer, pH 7.4 using a Potter-Elvehjem homogenizer at  $4^\circ\text{C}$  with a diluting factor of 4; the crude tissue homogenate was then centrifuged at a speed of 9000 rpm for 15 min in cold centrifuge; the

supernatant was kept at  $-20^\circ\text{C}$  for estimation of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) activities.

### Estimation of SOD, CAT and GSH (reduced glutathione) activities in liver and kidney homogenates

Superoxide dismutase (SOD, E.C. 1.15.1.1) in liver and kidney homogenates was measured using commercial kits (Randox Laboratories Ltd, UK). The activity was expressed as U/mg. One unit of SOD activity is the amount which causes a 50% inhibition of the rate of reduction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) under the conditions of the assay. Catalase (CAT, E.C. 1.11.1.6) activity was determined by using a commercial kit (Biovision K773-100). CAT activity was expressed as U/mg. One unit of catalase activity is the amount of catalase which decomposes 1.0  $\mu\text{mol}$  of  $\text{H}_2\text{O}_2$  per min at pH 4.5 and  $25^\circ\text{C}$ . Reduced glutathione (GSH, E.C. 1.8.1.7) was determined by a commercial kit (Randox Laboratories Ltd, UK) and expressed as mg/g.

All biochemical analyses were conducted by persons who were blind to treatment groups.

### Statistical analysis

Data were expressed as mean  $\pm$  SEM. Student's t-test was used to determine the difference between groups. Statistical significance was set at  $p < 0.05$ .

## RESULTS

### Effects of CHR aqueous extract on biochemical parameters

The serum levels of AST, ALT, ALP and GGT in control and experimental groups of rats are shown in Table 1. Oral administration of aqueous extract of *C. recutita* caused no significant change in serum levels of these hepatic enzymes when compared to the levels in control rats. Group 2 rats (alcohol only) developed hepatic response compared to group 1 (normal control). This was evidenced by a significant elevation ( $p < 0.05$ ) in the levels of all 4 hepatic enzyme markers. However, in animals given ethanol with pre- or post treatment with CHR extract, serum levels of all enzyme markers were significantly lower than their corresponding levels in Group 2 animals. The improvement in the levels of these enzymes was greater in the pretreated than in the post-treated group.

Also shown in Table 1, are levels of total protein, creatinine and urea in the serum of control and experimental groups of rats. In comparison to control group, oral administration of the aqueous extract of *C. recutita* resulted in normal levels of these biochemical parameters in the serum of the rats, while oral ethanol administration resulted in a significant decrease in total protein level and significant increases in serum levels of urea and creatinine. Changes in the levels of these parameters were reversed in ethanol administered rats pre-treated or post-treated with CHR extract with a greater reversal in pre-treated than in post-treated rats.

**Table 1.** Serum biochemical parameters of normal and experimental groups.

Biochemical parameter	Control	Ethanol	CHR	Pre-treated group	Post-treated
ALT (U/L)	81.81±6.05	152.66±3.61*	84.6±3.14	83.83±2.64**	105.8± 2.78**
AST (U/L)	165.80±4.14	213.85±2.99*	163.96±3.65	168.2±3.8**	177.18±2.09**
ALP (U/L)	86.40±4.16	166.5±6.22*	83.5±4.32	85.5±2.43**	105.33±2.56**
GTT (U/L)	7.18±0.26	9.15±0.67*	7.31±0.53	7.37±0.70**	7.05±0.70**
Urea (mg/dl)	46.8±3.03	78.3±5.13*	46.16±1.32	44.16±2.78**	56±1.78**
Protein (mg/dl)	7.36±0.39	4.6±0.27*	7.13±0.30	7.33±0.92**	6.25±0.15**
Creatinine (mg/dl)	0.414±0.030	0.536±0.025*	0.428±0.036	0.428±0.023**	0.491±0.094**

Values are mean ± SD, n=10. Ethanol and CHR treated rats were each compared to normal rats while pre-treated and post-treated rats were compared to ethanol treated rats. \*Significantly different at P < 0.05 when compared to controls; \*\*significantly different at P < 0.05 when compared to ethanol treated rats. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GTT, γ-glutamyltransferase.

**Table 2.** Hematological indices of normal and experimental groups.

Biochemical parameter	Control	Ethanol	CHR	Pre-treated group	Post-treated
RBC count (x 10 <sup>6</sup> /μl)	8.81±0.85	6.65±0.41*	8.97±0.64	8.45±0.51**	7.60±0.77**
Hb conc. (g/dl)	16.86±1.97	13.85±0.46*	16.27±0.57	16.96±0.30**	15.18±0.17**
Hct (PCV) (%)	46.06±3.28	42.62±0.55*	44.5±1.11	47.4±0.44**	43.75±5.4**
MCV (fl)	52.27±2.81	60.85±1.24*	49.47±1.18	53.3±1.05**	53.27±1.56**
MCHC (pg)	36.65±2.00	32.50±1.24*	36.25±1.32	35.7±0.93**	34.70±0.39**
RDW-SD (fl)	23.48±0.81	34.70±0.46*	23.18±0.70	24.33±0.92**	28.06±1.32**
Platelets (x 10 <sup>3</sup> / μl)	400.6±35.83	207.6±24.45*	373.8±47.1	382.2±37.10**	286.3±71.5**
TLC (x 10 <sup>3</sup> / μl)	17.04±0.99	9.07±0.99*	17.32±0.63	16.96±0.87**	13.85±0.55**

Values are mean ± SD., n=10. Ethanol and CHR treated rats were each compared to normal rats while pre-treated and post-treated rats were compared to ethanol rats. \*Significantly different at P < 0.05 when compared to control; \*\*significantly different at P < 0.05 when compared to ethanol treated rats. CHR, *Chamomilla recutita*; RBC, red blood cells; Hb.conc., hemoglobin; Hct, hematocrit, MCV, mean cell volume; MCHC, mean cell hemoglobin concentration; RDW, red cell distribution width; TLC, total leucocyte count.

### Effect of CHR aqueous extract on hematological parameters

As shown in Table 2, oral administration of ethanol to rats induced a significant decrease in total RBC count, hemoglobin concentration (Hb), packed cell volume (PCV) and mean hemoglobin cell concentration (MHCH) along with a marked increase in mean cell volume (MCV) and in the value of RBC distribution width (RDW-SD). Total leucocyte count (TLC) and platelets count were significantly decreased in the blood of ethanol treated rats. Oral administration of CHR aqueous extract alone did not cause any changes in the levels of these hematological parameters and kept them within the ranges found in normal control rats. On the other hand, ethanol treated rats pre-treated with the extract showed significant increases in RBC count, Hb, PCV, MHCH, platelets and leucocytes counts with significant decreases in MCV and RDW. A similar but weaker result was obtained in post-treated rats.

### Effect of the aqueous extract on oxidative stress parameters

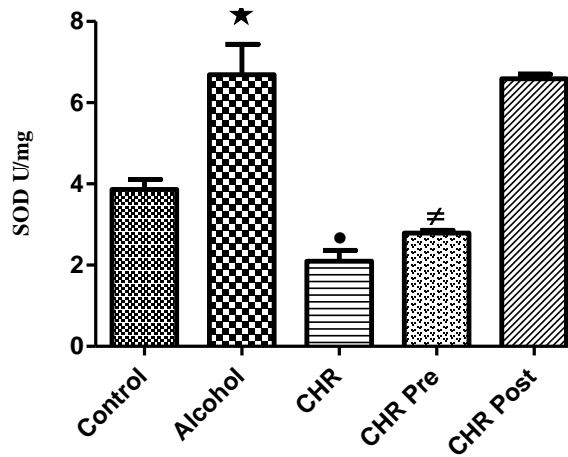
Activities of SOD and CAT as well as levels of GSH in

liver and kidney homogenates of control and treated rats are shown Figures 1 and 2. Ethanol intoxication resulted in statistically significant increases in the activities of SOD and CAT with significantly decreased levels of GSH in both liver and kidney compared to control rats. Pre-treatment with CHR extract resulted in significant reversal of ethanol induced changes in all 3 parameters in both liver and kidney tissues. The effect of post-treatment with CHR on ethanol treated tissues was similar with the notable exception that post-treatment did not significantly reverse the ethanol effect on GSH.

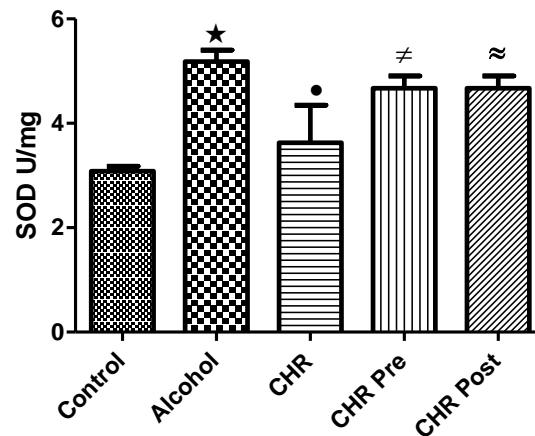
### DISCUSSION

The central role of the liver and kidney in drug metabolism and disposal of toxic material predisposes these organs to toxic injury. Nearly every drug has been associated with some degree of hepatotoxicity - almost certainly due to the vital role of the liver in drug metabolism. Hepatic metabolism is, first and foremost, a mechanism that converts drugs and other compounds into less potent but more easily excretable products (Sallie et al., 1999). However, a metabolite could have a higher activity and/or greater toxicity than the original drug. Metabolites

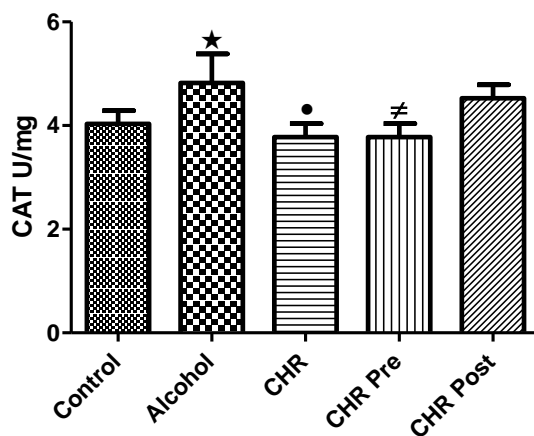
**SOD**



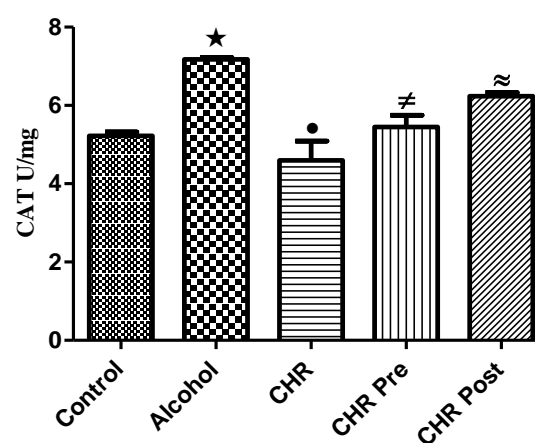
**SOD**



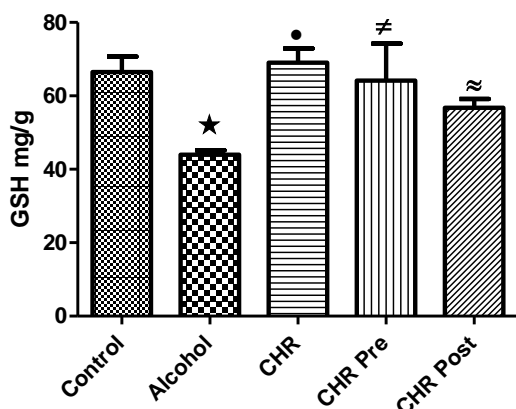
**CAT**



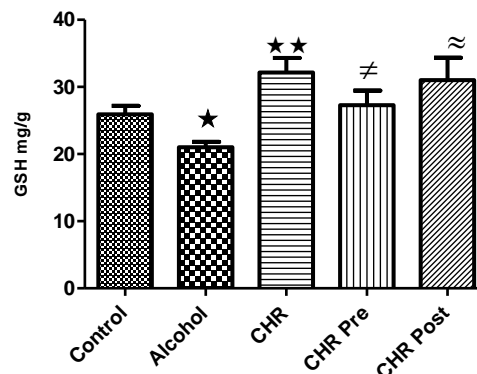
**CAT**



**GSH**



**GSH**



**Figure 1.** Oxidative stress parameters in livers of normal and experimental groups. Values are mean ± SD; n=10. \*, \*Significantly different at P< 0.05 when compared to controls; <sup>≠</sup>, <sup>≈</sup>, significantly different at P< 0.05 when compared to ethanol treated rats.

**Figure 2.** Oxidative stress parameters in kidneys of normal and experimental rats. Values are mean ± SD., n=10. \*, \*Significantly different at P< 0.05 when compared to controls; <sup>≠</sup>, <sup>≈</sup>, significantly different at P< 0.05 when compared to ethanol treated rats.

of drugs that are excreted from the kidney may cause cellular damage leading to kidney dysfunction (Singhal et al., 1998). In the present study, sub-acute ethanol administration (for a period of 28 days) significantly increased the levels of the hepatic enzymes - ALT, AST ALP. A rise in the AST level is usually accompanied by an elevated ALT level (Nyblom et al., 2004). The present results are in agreement with the clinical findings of Pari and Karthikesan (2007) who showed that chronic alcohol intake leads to many cellular and tissue abnormalities including alterations in liver enzymes (ALT, AST and ALP). These changes may indicate increased permeability, damage and/or necrosis of hepatocytes (Saravanan et al., 2006). In agreement with Ruppin et al. (1982) ethanol treatment, there was also a significant increase in the serum level of gamma-glutamyl transferase (GGT).

Oral administration of the aqueous extract of *C. recutita* to rats did not affect the levels of hepatic enzymes thus confirming the safe use of this plant extract on the liver. Both pre- and post-treatment of ethanol administered rats with aqueous CHR extract resulted in significant decreases in serum levels of these hepatic enzymes when compared to levels in ethanol treated rats. This effect was greater in pre- than in post-treated animals. Thus, CHR extract had a greater protective than therapeutic effect on hepatocytes against alcohol-induced damage and subsequent leakage of enzymes into the circulation.

Serum total protein level also monitors liver function. In the present study, ethanol treatment caused a significant decrease in serum total protein levels. This result is in line with that of Ahmed et al. (2002) who found a similar significant decrease in serum protein and albumin in ethanol-administered rats. Thus, ethanol impaired liver function and decreased hepatic protein synthesis. In the current study, significant increases in serum total protein levels were observed in CHR pre-treated and post-treated rats, an indication of the ability of CHR extract to restore normal liver function by stimulating regeneration of hepatic tissue and increased protein synthesis. Again, total protein levels were improved more in pre-than in post-treated rats.

Increased serum levels of urea and creatinine have been linked to kidney disease (Wheaton et al., 1994).

Urea is the main end product of protein catabolism. It represents 90% of the total urinary nitrogen excretion. In this study, ethanol treated rats exhibited significant increases in serum urea and creatinine pointing to a reduced ability of the kidney to excrete these products and impairment of kidney function. These effects may be attributed to the changes in the threshold of tubular re-absorption, renal blood flow and/or glomerular filtration rate (Kera et al., 1985). This is consistent with the findings of previous studies which reported ethanol-induced renal damage (Vamvakas, 1998).

Alcohol may also produce renal tissue damage by causing depletion of glutathione, mitochondrial damage,

deregulation of growth factor signaling and orchestration of cytokine-induced cellular injury (Molina et al., 2003). Oral administration of aqueous CHR extract to rats did not change the serum levels of creatinine or urea suggesting a safe uptake of the extract. Pre- or post-treatment with CHR extract of ethanol administered rats lowered the levels of these parameters in the serum with the greater effect shown in the pre-treated group. Again pretreatment with extract had a more potent protective than curative effect.

The current study shows that rats administered ethanol developed anemia. Administration of ethanol to rats for 28 days produced a significant bicytopenia in the form of moderate anemia and leucopenia. The anemia was macrocytic and hypochromic as shown by decreased total RBC count, decreased hemoglobin (Hb) and mean corpuscular hemoglobin (MCHC) concentrations, increased mean corpuscular volume (MCV) and increased levels of RBC distribution width (RDW-SD), indicating a degree of anisocytosis. Anemia may result from excessive RBC destruction/loss or decreased RBC production and is usually a manifestation of an underlying disease process (Beard et al., 1963). Increased RBC's hemolysis following acute or chronic ethanol administration has been ascribed to many factors. These include enhanced osmotic hemolysis due to increased erythrocyte fragility and impairment of the antioxidant defense mechanisms of the cell membrane, especially by changes in the lipid fluidity of the middle zone of the bilayer (Araki and Rifkind, 1981). Thus, decreased RBC count in ethanol treated rats might be a reflection of oxidative stress effects of ethanol on red cell membrane. Decreased RBC production may be due to bone marrow suppressive effect of ethanol (Ballard, 1980). Ethanol also suppresses marrow granulopoiesis (Hrelia et al., 1986). In this case, bone marrows are consistently hypocellular with marked decrease in mature granulocytes, and vacuolization of granulocyte precursors, similar to that of erythrocyte precursors (Sullivan and Herbert, 1964).

Oral administration of *C. recutita* aqueous extract to normal rats revealed the safe use of this plant on hematological parameters. The extract did not cause any change in any of the hematological parameters in the blood of the treated rats. On the other hand the extract showed protective and mild curative effects in ethanol administered rats. The results show a positive effect of this plant on total RBC's and WBC's count, Hb, PCV, Blood indices and RDW-SD. The possible mechanism responsible for the protective and curative effects of CHR extract in the observed ethanol-induced toxicity may be as a result of the extract acting as a free radical scavenger by intercepting radicals involved in ethanol metabolism by microsomal enzymes or the extract may contain phytochemicals. Certain flavonoids, triterpenoids and steroids are known to protect against toxins (Salmon, 1992). The presence of these compounds in *Chamomilla recutita* may be responsible for the protective effect on ethanol-indu-

induced tissue damage and hematological parameters in rats.

One previous study had implicated the generation of reactive oxygen species such as super oxide radicals, hydrogen peroxide and hydroxyl radicals in alcohol-induced renal tissue injury (Zurovsky and Haber, 1995). In this study, ethanol treatment resulted in significant increases in antioxidant enzymes SOD and CAT as well as a significant decrease in GTT, the enzyme which constitutes another antioxidant enzyme, glutathione. These changes were observed in both liver and kidney. Pre-treatment of ethanol administered rats with CHR significantly reversed all the above changes in both tissues; this reversal was more potent than those observed in post-treated rats. Thus, the results suggest diminution of anti-oxidant activity as one mechanism by which CHR extract protects and ameliorates ethanol-induced liver and kidney damage.

Some 120 chemical constituents have been identified in *C. recutita*, including terpenoids (chamazulene), tannins, flavonoids (apigenin and luteolin), and coumarins (umbelliferone, alpha-bisabolol). The flavonoids apigenin and luteolin possess anti-inflammatory, carminative, and antispasmodic properties (Salamon, 1992). The anti-inflammatory, wound-healing and antimicrobial effects of German chamomile are attributed to a blue essential oil that contains sesquiterpene alcohol, alpha-bisabolol, chamazulene, and flavonoids. Tannins or polyphenols have a number of physical and chemical properties in common, which underlie their physiological and pharmacological actions (antioxidant and radical-scavenging activities and their ability to complex with other molecules, such as proteins and polysaccharides). Vegetable polyphenols are known to inhibit lipid peroxidation in vitro and there is evidence of their ability to scavenge radicals, such as hydroxyl, superoxide and peroxy, which are important in cellular prooxidant states (Haslam, 1996).

In conclusion, *C. recutita* extract caused potent protective and mild reversible therapeutic effects against alcohol-induced liver damage and adverse effects on blood parameters. We suggest that *C. recutita* extract may be used to protect against toxic effects of chronic alcoholism on the liver and kidney systems. These results are compatible with the hypothesis that *C. recutita* exerts its protective and therapeutic effects by antioxidant and free radical scavenging actions. Further investigation of different phytoconstituents of *C. recutita* is needed to extend these findings.

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## REFERENCES

Ahmed B, Alam T, Varshney M, Khan AS (2002). Hepato-protective activity of two plants belonging to the *A. piaceae* and the *Euphorbiaceae* family. *J. Ethnopharmacol.* 79(3):313-316.

- Aliyu R. (1994). Biochemical evaluation of the medicinal properties of *Cochlospermum planchonii* root. Ph.D. Thesis. University of Jos, Nigeria.
- Al-Hashem F (2010). Gastroprotective effects of aqueous extract of *Chamomilla recutita* against ethanol-induced gastric ulcers. *Saudi Med. J.* 31(11):1211-1216.
- Amanvermez R, Demir S, Tunnel OK, Alvir M, Agar E (2005). Alcohol induced oxidative stress and reduction in oxidation by ascorbate/L-cys/L-met in the testis, ovary, kidney, and lung of rat. *Adv. Ther.* 22:548-558.
- Araki K, Rifkind JMA (1981). The rate of osmotic hemolysis: a relationship with membrane bilayer fluidity. *Biochim.Biophys.Acta.* 645: 81-90.
- Ballard HS (1980). Alcohol-associated pancytopenia with hypocellular bone marrow. *Am. J. Clin. Pathol.* 73:830-834.
- Beard JD, Barlow G, Tuttle A (1963). Observations of peripheral blood elements during chronic ethanol administration in dogs. *Physiologist* 6:163.
- Beauge F, Stibler H, Borg S (1987). Alterations of erythrocyte membrane organization in alcoholics. *Alcohol Alcohol. Suppl* 1:561-565.
- Burtis CA, Ashwood ER (1996). In: Teitz Fundamentals of Clinical Chemistry. 4th ed, NB Saunders Company, Philadelphia. pp. 312-335.
- Dahanukar SA, Kulkarni RA, Rege NN (2000). Pharmacology of medicinal plants and natural products. *Ind. J. Pharmacol.* 32:S81-S118.
- Fawcett JK, Scott JE (1960). A rapid and precise method for the determination of urea. *J. Clin. Pathol.* 13:156-159.
- Gonzalez-Quintela AR, Alende F, Gude J, Campos J, Rey LM, Meijide C, Fernandez-Merino C (2008). Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. *Clin. Exp. Immunol.* 151:42-50.
- Haslam E (1996). Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. *Journal of Natural Products.* 59: 205-215.
- Heemans EH (1998). Booze and blood: the effects of acute and chronic alcohol abuse on the hematopoietic system. *Clin. Lab. Sci.* 11(4):229-232.
- Herbert V, Zalusky R, Davidson CS (1963). Correlation of folate deficiency with alcoholism and associated macrocytosis, anemia, and liver disease. *Ann. Intern. Med.* 58: 977.
- Hrelia S, Lercker G, Biagi PL, Bordoni A, Stefanini F, Zunarelli P, Rossi CA (1986). Effect of ethanol intake on human erythrocyte membrane fluidity and lipid composition. *Biochem Int.* 12:741-750.
- Kera Y, Komura S, Ohbora Y, Kiriya T, Inoue K (1985). Ethanol induced changes in lipid peroxidation and nonprotein sulfhydryl content: Different sensitivities in rat liver and kidney. *Res. Com. Chem. Pathol. Pharmacol.* 47:203-209.
- Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. *J. Biol Chem.* 193:265.
- Madisch A, Melderis H, Mayr G, Sassini I, Hotz J (2001). A plant extract and its modified preparation in functional dyspepsia. Results of a double-blind placebo controlled comparative study. *Zeitschrift fur Gastroenterologie.* 39:511-517.
- Mahady GB, Fong HH, Farnsworth N (1999). *Floschamomillae*. WHO Monographs on Selected Medicinal Plants. Geneva, Switzerland: World Health Organization Publications.
- Mann C, Staba E (1986). The chemistry, pharmacology, and commercial formulations of chamomile. In: Craker LE, Simon JE (eds.). *Herbs, spices and medicinal plants: Recent advances in botany, horticulture, and pharmacology.* Arizona: Oryx Pressv1. pp. 235-280.
- Mills S, Bone K (2000). Principles and practice of phytotherapy. In: *Modern Herbal Medicine.* Edinburgh: Churchill Livingstone. p. 643.
- Molina PE, Happel KI, Zhang P, Kolls JK, Nelson S (2003). Alcohol and Health. Focus on Alcohol and the Immune System. National Institute on alcohol abuse and alcoholism. [pubs.niaaa.nih.gov/publications/arh40/97-108.htm](http://pubs.niaaa.nih.gov/publications/arh40/97-108.htm)
- Nordman R, Riviere C, Rouach H (1992). Implication of free radical mechanism in ethanol induced cellular injury. *Free Radic. Biol. Med.* 12:219-240.
- Nyblom H, Berggren U, Balldin J, Olsson R (2004). High AST/ALT ratio

- may indicate advanced alcoholic liver disease rather than heavy drinking. *Alcohol Alcohol.* 39(4):336-339.
- Ozaras R, Tahan V, Aydin S, Uzun H, Kaya S, Senturk H (2003). N-acetylcysteine attenuates alcohol-induced oxidative stress in rats. *World J. Gastroenterol.* 9:791-794.
- Pari L, Karthikesan K (2007). Protective role of caffeic acid against alcohol-induced biochemical changes in rats. *Fundamental Clin. Pharmacol.* 21(4):355-361.
- Ruppin DC, FrydmanMI, Lunzer MR (1982). Value of serum gamma-glutamyl transferase in the diagnosis of hepatobiliary disease. *MJA.* 1:421-424.
- Salamon I (1992). Chamomile, a medicinal plant. *Herb Spice Med. Plant Digest.*10:1-4.
- Sallie, R, Tredger JM, Williams A (1999). Drugs and liver biopharmacology. *Drug Dispos.* 12: 251-259.
- Saravanan R, Viswanathan P, Pugalendi KV (2006). Protective effect of ursolic acid on ethanol-mediated experimental liver damage in rats. *Life Sci.* 78:713-718.
- Singhal PC, Sharma P, Sanwal V, Prasad A, Kapasi A, Ranjan R, Franki N, Reddy K, Gibbons N (1998). Morphine modulates proliferation of kidney fibroblasts. *Kidney Int.* 53:350-357.
- Sullivan LW, Herbert V (1964). Suppression of hematopoiesis by ethanol. *J. Clin. Invest.* 43:2048-2062.
- Testino G (2008). Alcoholic diseases in hepato-gastroenterology: A point of view. *Hepatogastroenterology.* 55(82-83):371-7.
- United States National Institute for Health (1985). Publication No. 85. pp. 23.
- Vamvakas S, Teschner M, Bahner U, Heidland A (1998). Alcohol abuse: Potential role in electrolyte disturbances and kidney diseases. *Clin Nephrol.* 49:205-13.
- Voigt MD (2005). Alcohol in hepatocellular cancer. *Clin Liver Dis* 9(1): 151-69. doi:10.1016/j.cld.2004.10.003. PMID15763234. [http://journals.elsevierhealth.com/retrieve/pii/S1089-3261\(04\)00115-1](http://journals.elsevierhealth.com/retrieve/pii/S1089-3261(04)00115-1).
- Wheaton A, Watson AJ, Rock, RC (1994). Colorimetric determination of serum urea concentration. In: Burtis CA, Ashwood ER (eds). *Tietz Textbook of Clin. Chem.* WB Saunders Company London. Pp. 1528-1531.
- Watson WP, Little HI (2004). Alcohol and the brain. In: Drink, Drugs and Dependence: From science to clinical practice. Caan W, Bellerocche Jackie de (eds).Routledge. ISBN 978-04-1527891.
- WHO Global Report on Alcohol. (2004). Global Status on Alcohol. [http://www.faslink.org/WHO\\_global\\_alcohol\\_status\\_report\\_2004.pdf](http://www.faslink.org/WHO_global_alcohol_status_report_2004.pdf).
- Yeung K-Y, Klug PP, Lessin LS (1988). Alcohol-induced vacuolization in bone marrow cells. Ultrastructure and mechanism of formation. *Blood Cells.*13:487- 500.
- Zurovsky Y, Haber C (1995). Antioxidants attenuate endotoxin generation-induced acute renal failure in rats. *Scand. J. Urol. Nephrol.* 29:147-154.