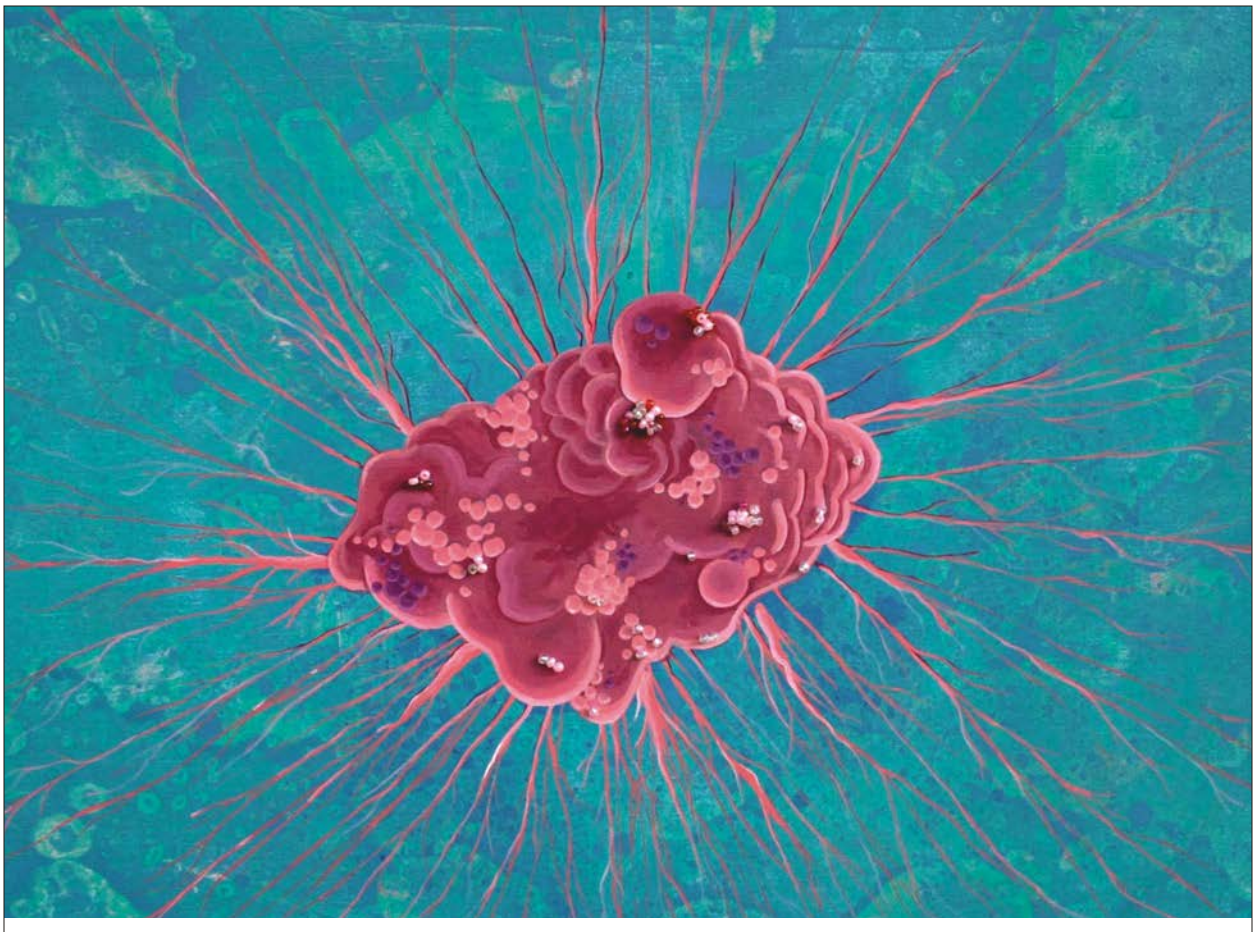

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Effect of *Catha edulis* on the Activities of Enzyme Markers of Carcinogenicity in Chemically-induced Hepatocellular Carcinoma in Rabbits

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ABSTRACT

Animals fed *Catha edulis* leaves develop an acute hepatitis and long-term feeding is associated with chronic active hepatitis and fibrotic liver disease. Repeated episodes of subclinical hepatitis with evolution to chronic liver disease has also been observed in patients chewing *Catha edulis* leaves. The aim of this study was to examine the effect of 10% *Catha edulis* on enzyme markers of carcinogenicity in relation to chemically-induced hepatocellular carcinoma in rabbits. Forty healthy male white New Zealand rabbits were allocated to one of five groups (eight rabbits per group). Two control groups fed on control diet with or without sodium nitrite+diethylamine, two treatment groups fed on a diet containing 10% *Catha edulis* with or without sodium nitrite in water and a fiveth group fed on diet containing tannin. Fasting blood samples were collected at different time intervals (1, 8 and 20 weeks) and plasma was assayed for γ-glutamyl transpeptidase, β-glucuronidase, LDH, AST and ALT using enzymatic kits. 10% *Catha edulis* alone did not affect these enzymes, however, animals maintained on 10% *Catha edulis* and sodium nitrite (4000 ppm) (58.82 mM) significantly increased the activities of γ-glutamyl transpeptidase, β-glucuronidase and LDH in a similar manner to those animals exposed to both carcinogens (nitrosamine precursors and commercial tannin). This raises the question of whether the *Catha edulis* hepatoxicity could be attributed to possible formation of nitrosamines *in vivo* from the secondary amines present in *Catha edulis* leaves; as well as highlighting the significance of these enzyme markers in early detection of chemically-induced HCC.

Key words: *Catha edulis*, khat, nitrosamin, tannins, hepatocellular carcinoma

INTRODUCTION

Hepatocellular Carcinoma (HCC) is considered to be among the leading causes of cancer-related deaths worldwide (Siegel *et al*., 2012; Chiappini, 2012; Jemal *et al*., 2011) and the most common primary cancer of hepatocytes (Davis *et al*., 2008; Somi, 2005). It is one of the most common life threatening solid tumors with global annual diagnosis exceeding one million new cases (Jemal *et al*., 2007). In developing countries, occurance of HCC represents more than 80% of cases.

Areas of particularly high incidence are Eastern and South-eastern Asia and Sub-Saharan Africa (Llovet *et al*., 2003). The occurrence and development of HCC is a complex multifactor and multistep process, mainly associated with chronic and persistent infection with the hepatitis virus, particularly the Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV), aflatoxin exposure (El-Serag, 2011; Hiotis *et al*., 2012) and in non-alcoholic fatty liver disease (Malaguarnera *et al*., 2009). The diagnosis of HCC is usually based on the atypical histopathology combined with the laboratory screening including index of hepatic damage, the index of cholestasis, the index of hepatic synthesis and finally, tumor markers and instrumental tests which include hepatic ultrasonography, Computed Tomography (CT), Nuclear Magnetic Resonance (NMR) and angiography (Malaguarnera *et al*., 2010).

The habit of *Catha edulis* Forsk (khat) chewing has prevailed for centuries among population in the horn of Africa and the Arabian Peninsula including the Yemen. Fresh leaves of *Catha edulis* are customarily chewed for its psychostimulatory effect (Al-Habori, 2005). The common adverse effects of *Catha edulis* are wide and variable (Al-Habori, 2005; Al-Motarreb *et al*., 2010); including psychoneurological disturbances such as neurosis (Hoffman and Al'Absi, 2010), vasoconstriction of coronary vasculature (Ali *et al*., 2010) as well as the still debated hepatotoxicity in humans (Chapman *et al*., 2010; Stuyt *et al*., 2011; Coton *et al*., 2011). However, khat-related hepatotoxicity has been demonstrated in animals (Al-Habori *et al*., 2002; Al-Mamary *et al*., 2002; Alsalahi *et al*., 2012) and the histopathologic changes in the liver resembles those induced in humans by ingestion of the drug ecstasy, another amphetamine-like compound (Jones and Simpson, 1999). It has been suggested that the high tannin content of khat leaves is responsible for the observed gastritis (Halbach, 1972) and the apparently observed high prevalence of oesophageal carcinoma in Yemen (Gunaid *et al*., 1995).

Catha edulis contains variable concentrations of primary amines such as cathinone, cathine and norephedrine (Geisshusler and Brenneisen, 1987) and secondary amines such as ephedrine and pseudoephedrine (Caveney *et al*., 2001) which may be considered to be precursors of nitrosamines (potent carcinogens) in the presence of nitrite. In light of previous findings of nitrosamine formation from nitrosation of aqueous extracts of different types of *Catha edulis* leaves *in vitro* (Al-Mamary *et al*., 2006) and its possible involvement in the observed high incidence of esophageal and forestomach carcinomas in Yemen. The aim of this study was to examine the effect of 10% *Catha edulis* leaves (containing tannin at 2.8%) in the presence and absence of sodium nitrite on the liver enzyme markers of carcinogenicity in HCC (Ramakrishnan *et al*., 2007) with respect to the chemically-induced HCC by exposing the animals to two carcinogens: nitrosamine precursors (diethylamine and sodium nitrite) and tannin and to evaluate the significance of these enzymes in the early detection of chemically-induced HCC. These enzyme markers include: γ-glutamyl transpeptidase (cell membrane), β-glucuronidase (lysosomal), lactate dehydrogenase (cytosolic), as well as aspartate aminotransferase (80% mitochondrial) and alanine aminotransferase (cytosolic).

MATERIALS AND METHODS

Catha edulis Forsk leaves (Sotty) were obtained from the local supplier and a voucher specimen was deposited in the Pharmacognosy department. The leaves were washed, dried and grounded before its added to the diets. Tannin was perchased from BDH Merck Ltd., UK.

Experimental design: Forty healthy male white New Zealand rabbits, weighting 800-1000 g were caged individually and given water and unpelleted food *ad libitum*. Rabbits were allocated

Table 1: The ingredients and nutrients composition of experimental diets

Ingredients (%)	Control	10% *Catha edulis*	2.8% Tannin
Corn	30	54	27.2
Soybean	8	5	8
Wheat bran	7	6	7
Wheat	25	0	25
Concentrate	10	10	10
Catha eduils	0	10	0
Sorghum	20	15	20
Tannin	0	0	2.8
Nutrient composition (% Dry matter)			
Crude protein	16.4	16.1	16.2
Crude fiber	11.4	10.4	11.0
kcal kg^{-1} ME	2680	2580	2610

to one of five groups (eight rabbits per group) (Table 1) and all diets were formulated according to Cheeke *et al.* (1987). The whole procedure had been approved by the IRB (Institutional Review Board) of the Faculty of Medicine and Health Sciences, Sana'a University.

- **Group 1:** Fed on a control diet
- **Group 2:** Fed on a diet containing 10% *Catha edulis* leaves
- **Group 3:** Fed on a diet containing 10% *Catha edulis* leaves (containing 2.8% tannin) and maintained on water contain sodium nitrite (4000 ppm) (58.82 mM)
- **Group 4:** Fed on a diet containing commercially perchased condensed Tannin at 2.8%
- **Group 5:** Fed on a control diet and maintained on water containing sodium nitrite (4000 ppm) (58.82 mM) and diethylamine (2000 ppm) (27.4 mM) for the induction of hepatoma

Fasting blood samples were collected at different time intervals (1, 8 and 20 weeks) after an overnight fast of 16 h. Blood was withdrawn from the marginal ear vein into EDTA tubes and samples were immediately centrifuged for 5 min at 2500 rpm and the separated plasma was stored in aliquots at -20°C. Plasma was assayed for γ-glutamyl transpeptidase (Sigma chemical Co., St. Louis, MO, USA), β-glucuronidase (Sigma chemical Co., St. Louis, MO, USA), lactate dehydrogenase (Randox), aspartate aminotransferase (Randox) and alanine aminotransferas (Randox) by using enzymatic kits.

Estimation of tannin: The estimation of tannin in dried *Catha edulis* leaves was carried out by the Vanillin-HCl reagent for condensed tannins. The vanillin reagent is prepared by combining equal volumes of 8% concentrated HCl in methanol and 2% vanillin in methanol. Polyphenolic material of the *Catha edulis* leaves was extracted by the method of Burns (1971) as modified by Maxson and Rooney (1972).

Statistical analysis: Samples were measured in duplicates and were expressed as Means±SD. Statistical analysis was carried out by Epi Info version 6 for Windows (Centers for Disease Control and Prevention, Washington, DC) and the significance was analysed by independent sample t-test between groups. Significant differences were considered at $p<0.05$.

RESULTS

Estimation of the condensed tannin as catechin equivalent in *Catha edulis* leaves used in this study was found to be 28 g 100 g^{-1} dried *Catha edulis*. Consequently, 2.8% commercial tannin was also tested which is equivalent to the amount of condensed tannin present in the 10% *Catha edulis* leaves.

Table 2 shows the effect of 10% *Catha edulis* with or without sodium nitrite drinking water, nitrosamine precursors and 2.8% commercial tannin on the activities of γ-glutamyl transpeptidase, β-glucuronidase and Lactate Dehydrogenase (LDH), after one week of treatment. Animals exposed to 10% *Catha edulis* leaves did not affect any of the enzymes tested; whereas those exposed to 10% *Catha edulis* with sodium nitrite drinking water had no effect on both γ-glutamyl transpeptidase and β-glucuronidase but had significantly ($p<0.01$) higher LDH (33.6%) activity. Similarly, tannin-fed animals had no effect on both γ-glutamyl transpeptidase and β-glucuronidase but had significantly ($p<0.01$) higher LDH (22%) activity. In contrast, those exposed to the nitrosamine precursors (diethylamine and sodium nitrite) had a significantly ($p<0.01$) higher levels of these enzymes by 78.6, 33.6 and 43%, respectively.

The changes in the activities of the above enzymes as well as Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) analyzed after 8 weeks of treatment are presented in Table 3. Animals exposed to 10% *Catha edulis* leaves did not affect any of the enzymes tested; whereas those exposed to 10% *Catha edulis* and sodium nitrite drinking water had significantly ($p<0.001$) higher γ-glutamyl transpeptidase (2.4 fold), β-glucuronidase (56%) and LDH (79.9%)

Table 2: Effects of *Catha edulis* (10%), *Catha edulis* (10%) plus $NaNO_2$, nitrosamine precursors and tannin (2.8%) on plasma activities of γ-glutamyl transpeptidase, β-glucuronidase and LDH after one week of treatment

	Groups				
Enzymes	Control	*Catha edulis*	*Catha edulis*+$NaNO_2$	Tannin	Nitrosamine precursors
γ glutamyl transpeptidase (U mL^{-1})	1.4±0.55	1.8±0.45	1.8±0.45	2.2±0.75	2.5±0.55*
β-glucuronidase (U mL^{-1})	23.2±2.39	22.8±1.92	24.8±1.30	25.0±2.61	31.0±4.43*
Lactate dehydrogenase (U L^{-1})	117.8±13.59	140.6±36.60	157.4±27.90*	143.7±14.77*	168.5±6.63*

The study includes 40 male white New Zealand rabbits weighing 800-1000 g and was allocated to one of five groups (8 rabbits per group). The groups were fed on diets containing: control diet, 10% CE, 10% CE and water containing sodium nitrite (4000 ppm), tannins (2.8%) and control diet and water containing sodium nitrite (4000 ppm)+diethylamine (2000 ppm). Results are presented as Means±SD, *p<0.01; **p<0.001

Table 3: Effects of *Catha edulis* (10%), *Catha edulis* (10%) plus $NaNO_2$, nitrosamine precursors and tannin (2.8%) on plasma activities of γ-glutamyl transpeptidase, β-glucuronidase, LDH, AST and ALT after 8 weeks of treatment

	Groups				
Enzymes	Control	*Catha edulis*	*Catha edulis*+$NaNO_2$	Tannin	Nitrosamine precursors
γ glutamyl transpeptidase (U mL^{-1})	1.6±0.55	2.0±0.71	3.8±0.84**	4.8±0.75**	5.5±0.84**
β-glucuronidase (U mL^{-1})	26.8±1.64	27.6±0.90	41.8±5.26**	50.0±7.51**	53.5±4.59**
Lactate dehydrogenase (U L^{-1})	138.6±20.24	157.8±26.86	249.4±46.24**	668.3±21.60**	753.2±25.30**
Aspartate aminotransferase (U L^{-1})	19.4±4.51	17.2±6.14	23.4±6.35	43.3±4.76**	71.2±5.98**
Alanine aminotransferase (U L^{-1})	25.6±6.23	23.0±7.48	31.0±2.55	37.8±2.48**	57.2±6.37**

The study includes 40 male white New Zealand rabbits weighing 800-1000 g and was allocated to one of five groups (8 rabbits per group). The groups were fed on diets containing: control diet, 10% CE, 10% CE and water containing sodium nitrite (4000 ppm), tannins (2.8%) and control diet and water containing sodium nitrite (4000 ppm)+diethylamine (2000 ppm). Results are presented as Means±SD, *p<0.01; **p<0.001

Table 4: Effects of *Catha edulis* (10%), *Catha edulis* (10%) plus $NaNO_2$, nitrosamine precursors and tannin (2.8%) on plasma activities of γ-glutamyl transpeptidase, β-glucuronidase, LDH, AST and ALT after 20 weeks of treatment

	Groups				
Enzymes	Control	*Catha edulis*	*Catha edulis*+$NaNO_2$	Tannin	Nitrosamine precursors
γ glutamyl transpeptidase (U mL^{-1})	2.6±0.55	3.6±0.89	5.8±1.31**	10.6±1.14**	19.3±2.94**
β-glucuronidase (U mL^{-1})	29.0±1.58	30.6±1.11	57.2±4.58**	54.0±3.81**	73.3±2.11**
Lactate dehydrogenase (U L^{-1})	145.0±13.26	198.8±61.66	260.0±82.73**	767.0±43.24**	889.0±75.53**
Aspartate aminotransferase (U L^{-1})	26.8±5.45	21.2±4.92	25.8±5.50	69.0±8.94**	81.0±19.80**
Alanine aminotransferase (U L^{-1})	30.8±3.03	22.8±8.17	34.0±9.50	52.0±2.12**	69.7±21.93**

The study includes 40 male white New Zealand rabbits weighing 800-1000 g and was allocated to one of five groups (8 rabbits per group). The groups were fed on diets containing: control diet, 10% CE, 10% CE and water containing sodium nitrite (4000 ppm), tannins (2.8%) and control diet and water containing sodium nitrite (4000 ppm)+diethylamine (2000 ppm). Results are presented as Means±SD, *p<0.01; **p<0.001

without any effect on both AST and ALT activities. On the other hand, tannin-fed animals and animals supplied with drinking water containing nitrsamine precursors had significantly ($p<0.001$) higher γ-glutamyl transpeptidase (3 fold and 3.4 fold), β-glucuronidase (86.6 and 99.6%), LDH (4.8 fold and 5.4 fold), AST (2.2 fold and 3.7 fold) and ALT (47.7% and 2.2 fold) activities.

Table 4 highlights the activities of enzymes analysed at 20 weeks of treatment. In the same manner as that observed on week 8, animals exposed to 10% *Catha edulis* leaves did not affect any of the enzymes tested; whereas those exposed to 10% *Catha edulis* and sodium nitrite drinking water had significantly ($p<0.001$) higher γ-lutamyl transpeptidase (2.2 fold), β-glucuronidase (97.2%) and LDH (79.3%) without any effect on both AST and ALT activities. On the other hand, tannin-fed animals and animals supplied with drinking water containing nitrsamine precursors had significantly ($p<0.001$) higher γ-glutamyl transpeptidase (4.1 fold and 7.4 fold), β-glucuronidase (86.2% and 2.5 fold), LDH (5.3 fold and 6.1 fold), AST (2.6 fold and 3 fold) and ALT (68.8% and 2.3 fold) activities.

DISCUSSION

N-nitroso compounds are known hepatocarcinogenic agents and have been implicated in the etiology of several human cancers (Bansal *et al.*, 2005), possibly by altering the DNA structure, forming alkyl DNA adducts and inducing chromosomal aberrations and micronuclei in the liver (Erkekoglu and Baydar, 2010; Al-Rejaie *et al.*, 2009). Nitrosamines can be formed endogenously from nitrate and nitrite and secondary amines under certain conditions such as strongly acidic pHs of the human stomach (Jakszyn and Gonzalez, 2006). The results presented in this study show 10% *Catha edulis* not to affect any of the enzyme markers of carcinogenicity in chemically-induced hepatocellular carcinoma in animals. *Catha edulis* at this level had previously been shown to be the least hepatotoxic on prolonged exposure (Al-Mamary *et al.*, 2002; Al-Habori *et al.*, 2002). However, the combined effect of 10% *Catha edulis* and sodium nitrite in drinking water increased the activity of some of these enzymes (γ-glutamyl transpeptidase, β-glucuronidase and LDH) in some cases to levels comparable with that attained by carcinogens such as nitrosamine precursors and commercial tannin. This apparent increase in hepatotoxicity in the presence of sodium nitrate may infer the possible formation of endogenous nitrosamines generated by the action of nitrite which on entering the stomach and at low pH converted to nitrous acid which reacts with secondary

amines in *Catha edulis* to give nitrosamines; which is in line with our earlier *in vitro* study Cheeke *et al.* (1987) in which nitrosamines were formed from *Catha edulis* leaves extract under simulated gastric condition. Nitrite and nitrate ions are naturally occurring forms of nitrogen and are present in drinking water, in human diet (green vegetables) and as food preservative (McMullen *et al.*, 2005). Moreover, a significant increase of γ-glutamyl transpeptidase activity with no effect on AST and ALT activities has also been observed in rats exposed to 30mg/ kg body weight sodium nitrite (Dudka *et al.*, 1995), highlighting the high toxicity of sodium nitrite which may react with diamines present normally in the diets and produce small amounts of nitrosamine.

Catha edulis-related hepatotoxicity mechanism is unknown; however severe chronic active hepatitis and portoportal fibrosis have been described in rabbits fed long term with fresh *Catha edulis*, supporting a direct toxic effect from reactive *Catha edulis* metabolites or an immuno-allergic reaction to these (Al-Habori *et al.*, 2002). This is further highlighted by the recent association of *Catha edulis* chewing with severe liver injury in East Africans in the UK suggesting that long-term *Catha edulis* chewing leads to repeated episodes of immuno-allergic or idiosyncratic hepatitis leading to fibrosis and cirrhosis (Chapman *et al.*, 2010; Stuyt *et al.*, 2011). Drug accumulation has also been proposed since a high concentration of cathinone was detected in the liver of a patient 3 weeks after the patient's last use of *Catha edulis* (Chapman *et al.*, 2010). Cathinone, a sympaticomimetic alkaloid, has structural similarity with amphetamine and ecstasy which can be hepatoxic (Jones and Simpson, 1999). This study further raises the question of whether the observed *Catha edulis* hepatoxicity could be attributed to the formation of nitrosamines *in vivo* from the secondary amines present in *Catha edulis* leaves.

The differing results between the *Catha edulis* exposed group and those tannin-fed animals, though contains 2.8% of condensed tannin as estimated by the vanillin-HCL reagent, may suggest either that tannin is very toxic in its pure form since it has been shown to induce gene mutation and chromosomal abnormalities in mammalian and human cells (Carver *et al.*, 1983); or that the type of tannin in the *Catha edulis*, though of the condensed form, is different from the commercial tannin fed to the animals. The latter suggestion is further strengthened by the finding that *Catha edulis* contain epigallocatechin (Abdel-Sattar *et al.*, 1999) as well as polyphenolic (proanthocynidines) constituents that have emerged to play a role as anti-oxidants (Hagerman *et al.*, 1998; Koga *et al.*, 1999) and hence may possess cancer-preventing rather than cancer causing effects. Epigallocatechin-3-gallate has been reported to possess antiproliferative (Nihal *et al.*, 2005), antiangiogenic (Fassina *et al.*, 2004) activities as well as protecting cultured rat hepatocytes against hepatotoxin-induced cell injury (Kagaya *et al.*, 2002).

On calculating the amount of nitrosamine generated from the precursors, animals were being exposed to ~140 mg kg^{-1} body weight. This amount is much greater than have been used in the literature, so as to ensure faster onset of the pre-neoplastic stage. Previously, exposure to dinitrosamine at 10 mg kg^{-1} body weight demonstrated the onset of the pre-neoplastic stage after 8 days (Buchmann *et al.*, 1992; Braunbeck *et al.*, 1992) which was reported to last for 18-22 weeks before the development of HCC (Pugh and Goldfarb, 1992). Consequently, the selected enzyme markers in this study were followed for 20 weeks.

Several epidemiological studies have shown the associations between abnormally high liver enzyme levels and risks and mortalities of many diseases (Strasak *et al.*, 2008; Lee *et al.*, 2008). Recently, significant associations of elevated GGT with the risk of several cancers have been reported (Van Hemelrijck *et al.*, 2011) and were also suggested to be an independent predictor of the risk of developing HCC in HBV patients (Hann *et al.*, 2012). γ-Glutamyl transpeptidase has

also been shown to be correlated with both ALT and α-fetoprotein (AFP), suggesting that γ-glutamyl transpeptidase, as a single clinical serum marker, represents the state of liver and HCC simultaneously (Zhang *et al*., 2011). The results presented in this study also demonstrate the significance of these enzyme markers in the early detection of chemically-induced HCC in animals. Enzymes such as γ-glutamyl transpeptidase were observed to increase markedly and as early as one week of treatment in the animals exposed to nitrosamine precursors reaching ~7.4 fold increase at 20 weeks of treatment with respect to the control group, a finding which is in good agreement with earlier studies reporting γ-glutamyl transpeptidase activity to be 3-13 folds higher in human fetal liver and primary hepatoma than that of adult liver (Fujisawa *et al*., 1976). Similar findings have reported γ-glutamyl transpeptidase activities to significantly increase in hepatocytes at the pre-cancerous stage (Sells *et al*., 1979; Fiala *et al*., 1972) as well as in early pre-neoplastic rat liver foci and primary HCC (Brouillet *et al*., 1994). Moreover, exposure of rats to nitrosamine precursors increased the γ-glutamyl transpeptidase activity by 3 fold after 4 months of treatment. Other studies have also reported significant increase in γ-glutamyl transpeptidase activity during diethylnitrosamine treatment (Kovalszky *et al*., 1992; Sulakhe *et al*., 1992; Tsuda *et al*., 1992). In these experimental studies low single doses of diethylnitrosamine administered to an animal model resulted in pre-neoplastic liver which in turn raised γ-glutamyl transpeptidase activities.

Gamma-Glutamyl transpeptidase has been extensively studied in relation to hepatocarcinogenesis (Ikeda and Taniguchi, 2005; Zhou *et al*., 2006) activating pro-oncogenes or inactivating tumor suppressor genes initiated by carcinogens. High activity of γ-glutamyl transpeptidase appears to be a distinctive feature of at least chemically-induced rat hepatoma (Brouillet *et al*., 1994). Some evidence of a close connection between γ-glutamyl transpeptidase activation and chemical carcinogenesis was reported in rat liver. This elevation reflects the progress of carcinogenesis, since its activity correlates with tumor growth rate, differentiation and survival of the host (Koss and Greengard, 1982). It was further strengthened by the observations that γ-glutamyl transpeptidase levels during hepatocarcinogenesis correspond to the accumulation of macroscopic changes in rat liver (Fiala *et al*., 1972, 1976). Moreover, high γ-glutamyl transpeptidase activities have been found in fetal and neonatal rat liver cells suggesting a relationship between the increase of γ-glutamyl transpeptidase activity and the proliferation of non-differentiated "stem" cells (with high γ-glutamyl transpeptidase activity) whose differentiation has been sidetracked and whose development into mature hepatocytes (with low or no γ-glutamyl transpeptidase activity) has been prevented by carcinogens (Sells *et al*., 1979).

Plasma activity of β-glucuronidase was also significantly increased in animals exposed to nitrosamine precursors, reaching 2.5 fold at 20 weeks of treatment; which is in agreement with that observed in rats and mice (Ohta, 1991). These results can be explained by the fact that β-glucuronidase play an important role in the degradation of some glucosaminoglycans which increases in some types of hepatomas (Kupchella *et al*., 1981). Furthermore, β-glucuronidase activity was also observed to increase in serum of human patients with primary HCC (Ohta *et al*., 1992) which has been explained in terms of increased protein synthesis by tumor cells (Giardina *et al*., 1992).

Along the same line, the plasma activity of LDH was significantly increased in animals exposed to the nitrosamine precursors throughout the experimental period reaching 5.4 and 6.1 fold at 8 and 20 weeks of treatment; which is consistent with those observed in serum of patients with HCC as well as in human HCC cell line (El Mouelhi *et al*., 1987; Shen *et al*., 1999) and in rats treated with nitrosamine. Moreover, cytosolic LDH activity was found to increase significantly in

rat liver during exposure to diethylnitrosamine (Kisen *et al.*, 1993). LDH is a fairly sensitive marker of solid neoplasm (Lippert *et al.*, 1981) and many studies revealed increased LDH activity in various types of tumor (Cheeke *et al.*, 1987; Kamaraj *et al.*, 2007). The possible reason for elevated levels of LDH may be due to utilization of higher glucose in cancerous conditions which is the only energy producing pathway for the uncontrolled proliferating malignant cells. Increased glycolytic rate evaluated as lactate production (Fanciulli *et al.*, 1994) as well as increased LDH activity (Fujiwara *et al.*, 1997) were both found to be associated with a rapid growth of malignant tumours in patients with HCC. The increased activity of LDH is explained by the reported findings of c-MYC gene in various human and animal tumours which is able to activate the expression of LDH and increase lactate production. The expression of LDH was suggested to be necessary for their neoplastic phenotype (Shim *et al.*, 1997).

Serum ALT and AST are released from damaged hepatocytes into blood and their activities have been widely recognized as effective tools to detect liver diseases (Kim *et al.*, 2008). Recently, transaminase (AST or ALT) levels were suggested to be independent risk factors for HCC with a linear dose-response trend (Wen *et al.*, 2012). Our Analysis of the AST and ALT showed these enzymes to be significantly increased in animals exposed to the nitrosamine precursors (3 fold and 2.3 fold) and those fed with tannin (2.6 fold and 68.8%); with the ratio of AST: ALT being greater than 1. The activities of both of these enzymes are higher than those suggested by Chen *et al.* (1995) for diagnosis of HCC and are also consistent with the significant rise of AST and ALT activities in plasma of rats treated with diethylnitrosamine (Tu *et al.*, 1999) and in serum of patients with HCC (El Mouelhi *et al.*, 1987; Rocchi *et al.*, 1997). A positive correlation between AST and LDH activities has previously been suggested (Uno *et al.*, 1996).

In conclusion, this study (1) Raises the question of whether the observed *Catha edulis* hepatoxicity could be attributed to the possible formation of nitrosamines *in vivo* from the secondary amines present in *Catha edulis* leaves; (2) Highlight the significance of these enzyme markers in early detection of chemically induced HCC as evident by the marked increase of these enzymes as early as one week of treatment and in view of recent reports of the role of these enzymes as prospective predictors of HCC.

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