

Hepatoprotective effect of hydroethanol extracts of *Allium sativum* and *Carica papaya* on CCl₄- induced liver damage in rats

ABSTRACT

Plant-based products, believed to be less toxic have been employed as hepatoprotective agents in recent times. Polyherbal therapy is reported to have therapeutic benefits over single herb therapy. *Allium sativum* and *Carica papaya* are two known plants used traditionally for the treatment of liver related diseases. Thus, in this study, the hepatoprotective and antioxidant potential of hydroethanol extracts of *Allium sativum* cloves (Garlic) and *Carica papaya* (Pawpaw) leaves (singly and combined) against CCl₄-induced toxicity in rats was evaluated. Thirty male albino rats used were classed into six (groups A- F) groups of five rats each and treated (orally) for 14 days. Group A- rats + distilled water (negative control); Group B- rats + CCl₄ in olive oil (positive control); Group C- rats + 200 mg/ kg-day combined extract of *A. sativum* and *C. papaya* (1: 1 ratio); Group D- rats + 200 mg/ kg-day extract of *A. sativum*; Group E- rats + 200 mg/ kg-day extract of *C. papaya*; Group F- rats + 100 mg/kg bw of silymarin. CCl₄ induce hepatotoxicity was characterized by an upsurge (P<0.05) in serum Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Transaminase (ALT) activities, and total bilirubin. Also, CCl₄ administration led to a substantial reduction (P<0.05) of reduced glutathione (GSH), Glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities and an increase (P<0.05) in lipid peroxidation (MDA) in the liver tissue. However, treatment with hydroethanol extract of *A. sativum* and *C. papaya* prevented the CCl₄ induced lipid peroxidation and returned the altered marker enzymes of serum and antioxidant enzymes level to near normal. The extracts of both plants demonstrated significant antioxidant and hepatoprotective activity as reflected by the results. Comparatively, the combined extract gave the most promising antioxidant and hepatoprotective potential, which may be due to positive synergistic effects of both plants over either plant used singly, thereby vindicating the use of polyherbal remedy over a single herb.

Keywords: *Allium sativum*, *Carica papaya*, hydroethanol, hepatoprotective, carbon tetrachloride, silymarin.

Introduction

Liver, being a main location where metabolism occurs, plays a central role in the detoxification of several toxins consumed, or formed during the absorption of food materials (Rane *et al.*, 2016). Also, it plays a critical role in storage and

secretion. Thus, the body relies on the liver for the regulation, production, storage and secretion of proteins, chemicals, nutrients and for purification and clearance of toxins/unnecessary body substances (Arige *et al.*, 2017). Liver diseases are

Corresponding Author: I. O. Okoro

Department of Biochemistry, Faculty of Science, Delta State University, Abraka, Delta State, Nigeria

Email: israelik@yahoo.com



among the main causes of morbidity and mortality universally. Drug induced liver damage has been one of the very familiar causative factors that is of major clinical challenge. The symptoms of drug-induced hepatotoxicity are extremely variable, reaching from asymptomatic rise of liver enzymes to liver failure (Russmann *et al.*, 2009). Liver damage is always linked with cellular necrosis, upsurge in tissue lipid peroxidation, and reduction in tissue glutathione (GSH) levels. Also, there are elevated serum levels of several biochemical markers (serum glutamate pyruvate transaminase (SGPT/ALT), glutamate oxaloacetate transaminase (SGOT/AST) and alkaline phosphatase (Arige *et al.*, 2017).

Plant-based products (being less toxic) have been employed as hepatoprotective agents in recent times. Hence, continual search of plant varieties for new hepatoprotective ability has been an active area of research (Afaf *et al.*, 2008). Garlic (*Allium sativum* L.), is of the Liliaceae family, which originated from Central Asia. It is one of the earliest of cultivated plants (Londhe *et al.*, 2011), and one of the timeliest documented plants used in the management of diseases and preservation of health. Garlic is used in several countries/cultures as a food element due to its typical flavor and prospective health benefits. Its biological activities (antioxidant, antibacterial and antifungal properties) have also been reported, which result from its organosulfur and polyphenols (Shrestha *et al.*, 2016). Garlic contains a varied array of phenolic compounds like anthocyanins and phenolic acids (Phan *et al.*, 2019). It is also rich in minerals and vitamins. In Africa, especially in Nigeria, garlic is used in the treatment of respiratory tract infections, abdominal discomfort, otitis media and diarrhea. Similarly, ascorbic acid mixture with garlic has been said to shield the liver against Cd induced toxicity in albino mice (Kaur and Sharma, 2015). Several cultures have accepted the probable use of garlic for preclusion and management of diverse

diseases. Distinctive compounds found in garlic are believed to possess anti-microbial and anti-tumor effects and lessen the risk for cardiovascular disorders (Bayan *et al.*, 2014).

Papaya (*Carica papaya* L.), also known as pawpaw, is of the Caricaceae family involving 31 species in four genera. Papaya contains phytochemicals such as carotenoids, glucosinolate, alkaloids and phenolics. Others include Lycopene, Betacryptoxanthin Betacarotinoic acid, protocatechuic acid, Benzylisothiocyanate, chlorogenic acid, Benzylglucosinolate, Quercetin and caffeic acid (Huang *et al.*, 2009). The fruits, seeds, leaves, flower, juice, root and stem bark of the plant have been described to possess antimicrobial, antifungal, anthelmintic, antimalarial, hepatoprotective, male/ female antifertility, anti-inflammatory anti-cancer, hepatoprotection, antioxidant and anti-dengue properties (Karishna *et al.*, 2008; Nugroho *et al.*, 2017). The nephroprotective activity of *Carica papaya* plant on CCl₄ induced renal injured rats has been reported (Olagunjua *et al.*, 2009).

The practice of herb-herb combinations, otherwise known as polyherbal therapy has existed for several years, but the scientific proof of their therapeutic benefits is wanting in the literature. Also, hopeful effect of drug combination over a single drug in disease treatment has been reported (Karole, 2019). Similarly, the content of bioactive compounds from plants differ subject to the environmental factors, post-harvest conditions, agronomic conditions and maturity (Martins *et al.*, 2016). Therefore, this study was directed at comparing the hepatoprotective and antioxidant potential of hydroethanol extracts of *Allium sativum* and *Carica papaya* leaves (singly and combined) against CCl₄-induced toxicity in rats.

Materials and Methods

Drug and chemicals

Silymarin and Carbon tetrachloride (CCl₄) were purchased from Sigma chemicals, USA. Serum Glutamate Oxaloacetate Transaminase (SGOT/AST), Serum Alkaline Phosphatase (ALP), Serum Glutamate Pyruvate Transaminase (SGPT/ALT), Serum Total bilirubin (TB), Albumin and Total protein kits were purchased from RANDOX Laboratories Ltd., Ardmore, UK. All other chemicals for this study were of analytical grade.

Collection and preparation of plant materials

Carica papaya leaves were collected from Site II of the Delta State University, Abraka, while the garlic cloves were purchased from Abraka market and were both authenticated by Dr. H. A. Akinnobosun of the Department of Plant Science (Herbarium Unit), University of Benin, with voucher numbers: UBH_A388(Garlic) and UBH_C505 (Pawpaw). The samples were separately washed in running water tap, cut into tiny pieces, air dried and extracted with hydroethanol (ethanol: water, 50:50 v/v) as described by Okoro *et al.*, 2014. The extracts were kept at 4 °C before use. The dried extract was suspended in distilled water to prepare the concentration (200 mg/kg bw) used in this study.

Animals

The male rats (weighing 150 - 220 g) used for the study were bought from the Anatomy Department of Delta State University, Abraka, Nigeria. They were fed grower's mash (Top Feed, Ltd, from Sapele in Delta State) and water *ad libitum*. They were maintained in agreement with the guidelines on the care and wellbeing of research animals.

Grouping and treatment

The male albino rats were grouped into six (groups A- F) groups of five rats each and were treated orally for 14 days as follows:

Group A- Animals + distilled water only (negative control).

Group B- Animals + CCl₄ in olive oil vehicle only (positive control).

Group C- Animals + 200 mg/ kg-day combined extract of A. sativum and C. papaya (Mixture of both extracts in ratio of 1:1)

Group D- Animals + 200 mg/ kg-day hydroethanol extract of A. sativum.

Group E- Animals + 200 mg/ kg-day hydroethanol extract of C. papaya

Group F- Animals + 100 mg/kg bw of silymarin.

Treatment of the rats continued for 14 days and groups B – F animals were injected intraperitoneally with CCl₄ in olive oil vehicle at a dosage of (1 ml/kg bw) on the 14th day (30 min after the last treatment). The animals were fasted and sacrificed by cardiac puncture, 24 h after CCl₄ administration to obtain the serum and liver tissue used for the assays of this study.

Serum biochemical parameters determination

Serum samples were used for hepatic function tests using standard diagnostic kits (from RANDOX Laboratories Ltd., Ardmore, UK), by the following methods: Albumin (Gornall *et al.*, 1949), Total bilirubin (Trinder, 1969), Total protein (Jendrassik and Grof, 1938), alkaline phosphatase (Doumas *et al.*, 1971), alanine aminotransferase (Reitman and Frankel, 1957) and aspartate aminotransferase (Sood, 1999).

Oxidative stress markers determination

The following methods were used for the assays: The levels of antioxidants in liver tissue (SOD, CAT, GSH and GPx) were estimated by the method of Marklund (1992), Sinha (1972), Ellman (1970) and Rotruck *et al.* (1973) respectively. While, Lipid peroxidation byproduct [malondialdehyde (MDA)] in liver tissue was by Ohkawa *et al.* (1979).

Statistical analysis

Data are presented as mean \pm standard deviation and compared by the analysis of variance (ANOVA), and Turkey's post hoc test (for statistical significance) using GraphPad Prism (6.0 version). Significant level was at $p < 0.05$.

Results

Biochemical changes in serum parameters

The effects of hydroethanol extracts of *A. sativum* and *C. papaya* on liver functioning of CCl₄ intoxicated rats after 14 days treatment is shown in Figures 1-6. Single dose of CCl₄ administration significantly ($p < 0.05$) increased the levels of ALT, ALP and AST in the positive control group, compared to the negative control group. Also, a significant ($p < 0.05$) increase in the level of TB was seen in the positive control animals compared to the negative control group. On the other hand, pre-treatment with either the extract or the standard drug significantly ($p < 0.05$) reduced the levels of hepatic enzymes of serum (ALT, AST and ALP) and TB, when compared with the positive control group. The reduction in hepatic serum enzymes were in the following order: F > C > D > E (ALT); C/F > D > E (AST); F > C > E > D (ALP); F > C/D/E (TB). However, lower level of albumin and total protein were noticed in the positive control rats, compared with the negative control group of rats. While significantly ($p < 0.05$) higher levels of these two parameters were seen in the rats given either extracts or the standard drug, with the combined extract group (D) showing values for albumin and total protein near normal.

Superoxide dismutase (SOD) and catalase (CAT) activities

The activity of SOD in liver tissue homogenate was significantly decreased in CCl₄ treated animals, compared with negative control. However, pretreatment with both extracts and the

standard drug showed significant increase in SOD activity in all treated groups (Figure 7). Similarly, the CAT activity in CCl₄ treated rats (positive control) was significantly ($p < 0.05$) decreased when compared to negative control group. Again, pretreatment with both extracts significantly restored the CAT activity (Figure 8). Similarly, treatment with the standard drug appreciably increased the catalase activity (Figure 8).

Lipid Peroxidation (LPO) level, Glutathione (GSH) and Glutathione Peroxidase (GPx) Activities

Effect of *A. sativum* and *C. papaya* extracts on LPO were measured by the formation of free MDA in the liver tissue following exposure to CCl₄, and shown in Figure 9. Upon CCl₄ administration, MDA levels increased significantly ($p < 0.05$) in the positive control rats. However, treatment with either extract or the standard drug significantly inhibited the formation of MDA in the treated groups. On the other hand, a significant ($p < 0.05$) decrease in GSH concentration was observed in CCl₄ treated rats (positive control) compared with the negative control group. Administration of the hydroethanol extract of *A. sativum* and *C. papaya* for 14 consecutive days afforded a protection against GSH depletion in the treated groups (Figure 10).

The activity of Glutathione Peroxidase in the different treated groups of rats is shown in Figure 11. Significant decrease ($p < 0.05$) in GPx activity was observed for the CCl₄ treated rats, while treatment with the *A. sativum* and *C. papaya* extract caused a significant increase ($p < 0.05$) in the extract treated rats when compared with the CCl₄ treated rats. Similarly, a significant increase ($p < 0.05$) was also observed in the silymarin treated groups compared with the positive control group (Figure 11).

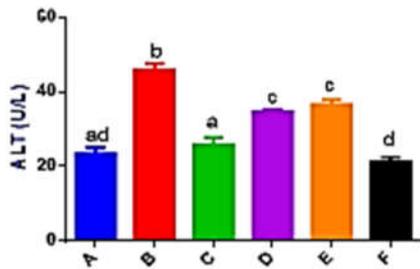


Figure 1: Effects of hydroethanol extracts (*A. sativum* and *C. papaya*) and silymarin on alanine aminotransferase (ALT) activity

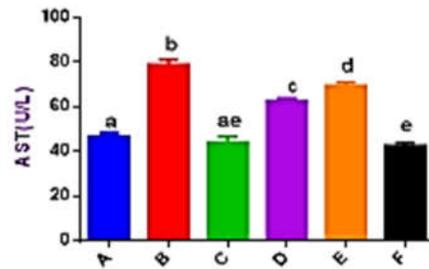


Figure 2: Effects of hydroethanol extracts (*A. sativum* and *C. papaya*) and silymarin on aspartate aminotransferase (AST) activity

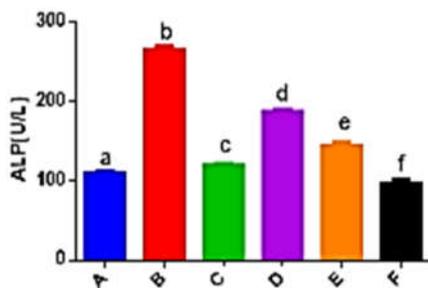


Figure 3: Effects of hydroethanol extracts (*A. sativum* and *C. papaya*) and silymarin on alkaline phosphatase (ALP) activity

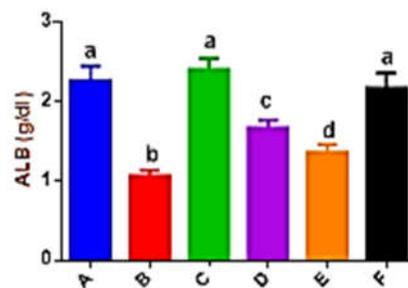


Figure 4: Effects of hydroethanol extracts (*A. sativum* and *C. papaya*) and silymarin on Albumin (ALB) level

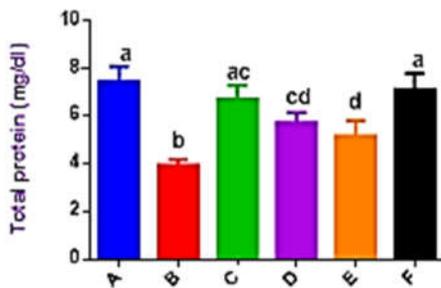


Figure 5: Effects of hydroethanol extracts (*A. sativum* and *C. papaya*) and silymarin on Total protein (TP) level

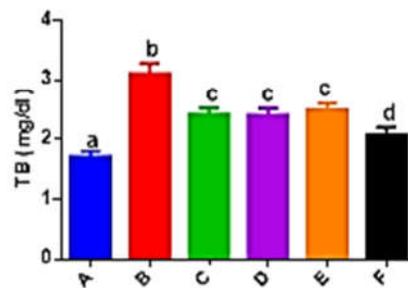


Figure 6: Effects of hydroethanol extracts (*A. sativum* and *C. papaya*) and silymarin on Total bilirubin (TB) level

*Bars with unlike letters differ considerably ($p < 0.05$). ** Keys : A= rats + distilled water (negative control); B = rats + CCl₄ in olive oil (positive control); C = rats + 200 mg/ kg-day combined extract of *A. sativum* and *C. papaya* (1: 1 ratio); D = rats + 200 mg/ kg-day extract of *A. sativum*; E = rats + 200 mg/ kg-day extract of *C. papaya*; F = rats + 100 mg/kg bw of silymarin.

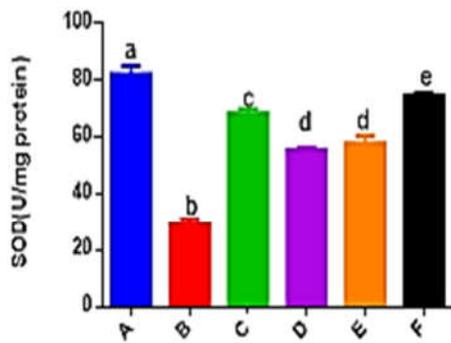


Figure 7: Effects of hydroethanol extracts (*A. sativum* and *C. papaya*) and silymarin on Superoxide dismutase (SOD) level

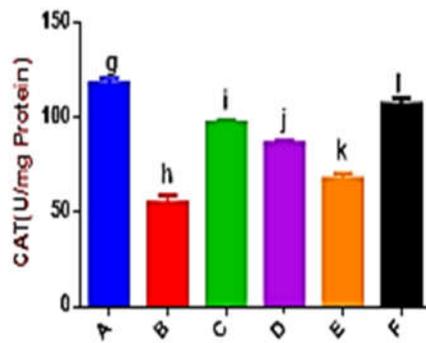


Figure 8: Effects of hydroethanol extracts (*A. sativum* and *C. papaya*) and silymarin on Catalase (CAT) level

*Bars with unlike letters differ considerably (p<0.05) ** Keys : A= rats + distilled water (negative control); B = rats + CCl4 in olive oil (positive control); C = rats + 200 mg/ kg-day combined extract of *A. sativum* and *C. papaya* (1: 1 ratio); D = rats + 200 mg/ kg-day extract of *A. sativum*; E = rats + 200 mg/ kg-day extract of *C. papaya*; F = rats + 100 mg/kg bw of silymarin.

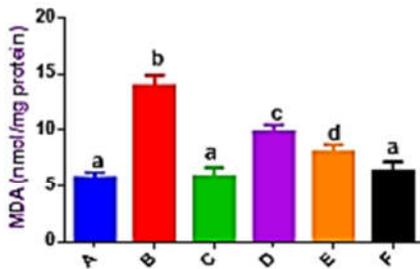


Figure 9: Effects of hydroethanol extracts (*A. sativum* and *C. papaya*) and silymarin on Malondialdehyde (MDA) level

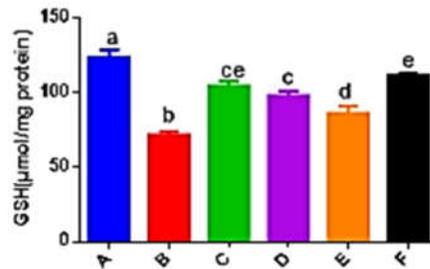


Figure 10: Effects of hydroethanol extracts (*A. sativum* and *C. papaya*) and silymarin on Glutathione (GSH) level

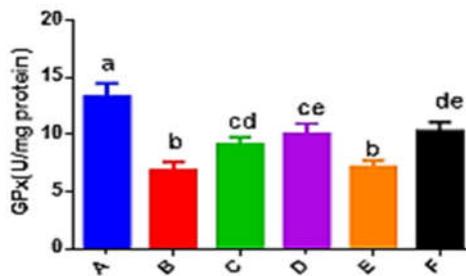


Figure 11: Effects of hydroethanol extracts (*A. sativum* and *C. papaya*) and silymarin on Glutathione peroxidase (GPx) activity

*Bars with unlike letters differ considerably (p<0.05) ** Keys: A= rats + distilled water (negative control); B = rats + CCl4 in olive oil (positive control); C = rats + 200 mg/ kg-day combined extract of *A. sativum* and *C. papaya* (1: 1 ratio); D = rats + 200 mg/ kg-day extract of *A. sativum*; E = rats + 200 mg/ kg-day extract of *C. papaya*; F = rats + 100 mg/kg bw of silymarin.

Discussion

The defensive roles of certain herbal extracts on intoxicated liver cells have been reported (Ajiboye *et al.*, 2010; Bag and Mumtaz, 2013). Amongst xenobiotics, CCl₄ is a key causative agent of severe liver cell injury through free radical generation (Guo *et al.*, 2017). In this study, the hepatoprotective and antioxidant potential of hydroethanol extracts of *Allium sativum* and *Carica papaya* leaf (singly and combined) were compared against CCl₄-induced toxicity in rats.

Aspartate aminotransferase (AST) is mostly located in mitochondria of hepatocytes. ALT is specifically found in the liver, and hence a better parameter for identifying liver injury. Also, ALP and bilirubin is connected with liver injury (Girish *et al.*, 2009). Results of this experiment show an increase in the serum levels of ALT, ALP, AST and TB in the positive control group when compared to the negative control group, following a single dose of CCl₄ administration. This observation agrees with earlier reports that administration of CCl₄ led to a significant increase in the levels of AST, ALP and ALT, which may be ascribed to destruction of the structural integrity of liver, since they are localized in the cytoplasm and must have been discharged into circulation following cellular damages signifying occurrence of hepatotoxicity (Gutiérrez and Solís, 2009). But, pretreatment with either extracts or the combined extract caused an appreciable reversal of the CCl₄ induced alteration of the liver marker enzymes, with the combined extract giving the best result among the extracts. During liver damage, cellular enzymes like ALT, ALP and AST present in the hepatic cells, escape into the serum leading to a surge in their concentration. The decrease in raised serum levels of the liver marker enzymes following extract treatment in groups C – E may be partly due to the protective effect of *Allium sativum* and *Carica papaya* plants extracts on

hepatocytes following the renewal of the liver cell membrane permeability (Ghanbari *et al.*, 2016). This protective effect shown signifies a decrease in enzymes existing in the liver cell's extra cellular milieu (Sarkar and Sil, 2007).

In this study, the administration of CCl₄ led to a decrease in the albumin and total protein levels in the positive control group relative to the negative control/normal rats. A decline in total protein may be as a result of disruption and severance of polyribosome on endoplasmic reticulum heading to flawed protein biosynthesis (Dubey *et al.*, 1994). Pretreatment with extracts of either plant improved the serum ALB and TP levels with variable degree of significance in groups C – E, the best result being obtained from the combined extract group. This observed improvement may be as a result of the elevation of the assemblage of ribosome on endoplasmic reticulum to expedite continuous protein biosynthesis (Sabbani *et al.*, 2016).

The liver and kidney are the primary focus organs of CCl₄ toxicity, and once CCl₄ is metabolically activated, then free radical reactions are started resulting in oxidative stress and subsequently peroxidation of cellular lipids (Tirmestein *et al.*, 2007). The CCl₄ action result from its free radical transformation to a trichloromethyl radical which binds to macromolecules in liver cells membrane. Glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) are important enzymes vital in the enzymatic antioxidant defense system, where they act to scavenge hydrogen peroxide and superoxide ion and defend the cells against lipid peroxidation. These antioxidant enzymes counteract the production of hydroxyl radical and shield the cellular components from oxidative damage (Scott *et al.*, 1991).

In this study, the administration of CCl₄ to animals resulted to an increase in liver tissue MDA level. Also, the activities of SOD, CAT,

GPx and GSH in the liver tissue homogenate were significantly decreased in CCl₄ treated animals (group B), compared with negative control (group A). Glutathione is a common tripeptide found in the liver. It is mainly concerned with the elimination of free radical species like alkoxy radicals, hydrogen peroxide and superoxide radicals and also acts as substrate for glutathione peroxidase (Prakash *et al.*, 2001). A decreased level of GSH observed in this study was related with an increased lipid peroxidation in the CCl₄-treated rats. However, pretreatment with either extracts or the combined extract showed a great capability of the extracts to decrease oxidative stress by normalizing SOD and CAT levels. Similarly, the glutathione and MDA levels were enhanced by treatment with extracts of both plants, thereby precluding lipid peroxidation. Thus, both plants extract possess antioxidant and hepatoprotective effect by regulatory action on suppressing oxidative stress and cellular permeability. Previous findings signified that specific steroids, flavonoids and triterpenoids possess hepatoprotective effect due to their antioxidant properties. Phenolic compounds are known to be the most significant antioxidative constituents of herbs/plant materials (Gupta and Misra, 2006).

A good relationship between the total antioxidant capacities and the concentrations of plant phenolic has been reported (Pellegrini *et al.*, 2000). Phytochemically, *C. papaya* contains carotenoids, glucosinolate, alkaloids, phenolics, Lycopene, Betacryptoxanthin Betacarotinoïd, protocatechuic acid, Benzylisothiocyanate, chlorogenic acid, Benzylglucosinolate, Quercetin and caffeic acid (Huang *et al.*, 2009), while the presence of phenolic compounds like anthocyanins and phenolic acids, have been reported for *A. sativum* (Bozin *et al.*, 2008; Phan *et al.*, 2019). In this study, both extracts used, either singly or combined demonstrated significant antioxidant and hepatoprotective activity as reflected by the results for serum

hepatic marker enzymes and the liver tissue antioxidant parameters, with the combined extract giving the most promising antioxidant and hepato-protective capacity. Therefore the observed effect may be due to positive synergistic effect of both plants over either plant used singly (Karole, 2019), and may also be attributed to the phytoconstituents of the extracts, which agrees with earlier reports on the antioxidant and hepatoprotective properties of *A. sativum* (Kaur and Sharma, 2015; Shrestha *et al.*, 2016) and *C. papaya* (Nugroho *et al.*, 2017).

Conclusion

The induction of liver damage by CCl₄ in this study was characterized by a rise in tissue lipid peroxidation and reduction of reduced glutathione levels. Also, serum levels of various biochemical markers such as ALT, ALP and AST were raised above normal. However, treatment with either extract of *A. sativum* / *C. papaya* or the combine extract at 200 mg/kg,bw significantly reduced the raised serum levels of the markers enzymes and prevented the CCl₄ induced lipid peroxidation and returned the antioxidant enzymes level to near normal. The results have shown the hepatoprotective activity of the extracts with the combined extract exhibiting a more promising activity overall among all extracts.

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