Al Ameen J Med Sci 2019;12(3):127-133 ● US National Library of Medicine enlisted journal ● ISSN 0974-1143

ORIGINAL ARTICLE CODEN: AAJMBG

Evaluation of comparative hepatoprotective activity of organic versus non-organic *Camelia sinensis* methanol extracts in albino mice

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Received: 15th March 2019; ***Revised*:** 25th May 2019; ***Accepted:*** 18th June 2019; ***Published:*** 01st July 2019

Abstract: *Objectives:* The study was carried out to compare hepatoprotective activity of organic versus non organic *Camelia sinensis methanol extracts in albino mice. Materials and Methods*: In vivo hepatoprotective activity of methanol extracts of organic and non-organic *Camelia sinensis* extracts (200 and 400 mg/kg) were evaluated using experimental CCl_4 induced toxicity model. The mice were divided into seven group (n=6). The mice were administered once daily with 10% DMSO (negative control), 25 mg/kg Silymarin (positive control), organic tea methanol extracts (200mg/kg, 400mg/kg) and nonorganic tea methanol extracts (200mg/kg, 400mg/kg) for seven days followed by the hepatotoxicity induction using carbontetrachloride. The blood samples were collected and subjected to biochemical analysis. In addition, histopathological examination of the hepatocytes was performed to observe any damage in a cellular level. Ballooning degeneration, cytic necrosis, confluent necrosis, submassive necrosis, micro and macro vesicular steatosis, lymphocyte infiltration were observed in the treatment and CCl_4 induced toxicity model. *Results*: Severe hepatic damage due to CCl_4 was indicated by significant increase in the liver enzymes level including Aspartate Aminotransferase (AST), Alanine Aminotransaminase (ALT) and Alkaline Phosphatase (ALP). The elevated levels of these enzymes were reduced in a dose-dependent manner in biochemical analysis by the organic and nonorganic tea methanol extracts. The reduction of liver enzymes level is better in organic treated mice than that of non-organic treated mice. Mice injected with CCl_4 have ballooning degeneration with confluent necrosis along with mixed infiltration of inflammatory cells which represents that the liver is highly injured. However, the organic extracts treated mice showed potent hepatoprotective effect with minimal focal ballooning degeneration indicating that the hepatocytes morphology resembling towards normal. Interestingly, the non-organic extracts showed a hepatoprotective effect but there are massive micro-vesicular steatosis and focal lymphocytes. We found a superior hepatoprotective activity of organic extracts than that of non-organic extracts. The liver injury produced by CCl_4 has been reversed by the Silymarin which is the positive control in the experiment. *Conclusion:* The study revealed that the hepatoprotective activity of organic *Camelia sinensis* is better than the non organic *Camelia sinensis*.

Keywords: *Camelia sinensis,* Hepatoprotective activity, Organic tea, Biochemical analysis, Hepatotoxicity, Carbontetrachloride

Introduction

The crude plants have gain popularity for the treatment of liver disease. It has been claimed and reported that 600 commercial and reported formulations containing hepatoprotective activity sold all over the world. There are small number of plants used in traditional medicine for hepatoprotective effect which are evaluated pharmacologically for their safety and efficacy. Most of the plants in Nepal are also not explored for their scientific medicinal uses. Thus, the hepatoprotective activity of *Camelia sinensis* was investigated through carbon tetrachloride induced hepatotoxicity in rats [1-3]. Liver disease, which is caused by the action of free radicals through the

mechanism of covalent binding and lipid peroxidation with subsequent tissue injury, is a global serious health problem. Hence, the plants that possess antioxidant properties are of concerns for preventing liver disease [4]. *Camelia sinensis* contains phenolic compounds which are responsible for the antioxidant effects [5]. The strong antioxidant properties are due to EGCG and EGC [6].

The polyphenols are mainly comprised of catechin and catechin derivatives including (-) epigallocatechins(EGC), (-)epigalocathechin-3-gallate (EGCG), (-)-1-epicatechin (EC), (-)-epicatechin gallate (ECG) and (-)-gallocatchin gallate (GCG). Recently, research focus on green tea is relatively increasing due to potential antioxidant activity of EGCG. Furthermore, literature has shown that EGCG arrest the progression of fibrosis and prevents the induced liver damage in animal model by inhibiting oxidizing damage [7-10]. The total phenolic content of organic tea is greater than that of non organic tea which is responsible for antioxidant properties and hepatoprotective effects [11]. Furthermore, as per the research, the fluoride content differs with the change in variety of tea. Generally, the fluoride content is less in organic tea [12].

Based on the fertilizers used, tea is divided into two groups:

1. *Organic tea:* Organic tea is free from synthetic fertilizer, herbicide and pesticides. There are many organic tea farming in Darjeeling- eastern region of Nepal like Ilam, Dhankuta. Due to decreased export and price of Non organic tea in international market, the farmers in Darjeeling and some part of Ilam are attracted towards cultivating organic tea. Natural fertilizer is used instead of synthetic fertilizer to increase productivity. The natural fertilizer used includes Cowdung, Goatdung, Cattle urine spray etc.
2. *Nonorganic tea:* Nonorganic tea is enriched with the numbers of synthetic fertilizer, pesticide and herbicide. Nonorganic tea is found widely in the eastern part of Nepal. Different synthetic fertilizer and pesticides are used to increase the productivity of the cultivation. Most of the farmers are using the fertilizer, pesticide in huge amount without the knowledge of its use hoping for greater production and economy. This is the reason considered for decreasing the expert quality of tea. The fertilizers used in nonorganic tea include Monochrotphus (Luphos 36), glycophosate (Roundup), Urea, Phosphorous etc [13].

Material and Methods

The study was carried out after the approval of Institutional Review Committee, UCMS Bhairahawa. The approval number is UCMS/IRC/064/15. The fresh leaves of *Camelia sinensis* were collected from Samalbung, Ilam eastern part of Nepal. The collected green leaves were washed in the tap water. Afterwards, the washed leaves were shade dried at room temperature (26 degree Celsius) for 10 days. The dried leaves were mechanically crushed into small pieces and packed in the air tight polythene bags.

Mechanically crushed dried leaves packed in air tight polythene bags were transferred in pharmacognosy lab and the extraction was carried out by maceration using methanol in the ratio of 1:□20 (w/v) for 72 hours, and the supernatant was filtered sequentially using Whatman no. 1 filter paper. The solvent was then evaporated using a vacuum rotary evaporator (Buchi Rotavapor, S331 India) under reduced pressure (204□mbar) and controlled temperature (40°C). The residue was collected and transferred to the air tight container for the experimental analysis.

Hepatoprotective activity of methanol extracts:

Animal model: Adult wister albino mice weighing 25-30 g of either sex bred in Department of plant resources, Thapathali, Kathmandu were used for the study. They were maintained in standard housing condition in Universal College of medical sciences (UCMS), Department of pharmacology. The mice were fed with the water ad libitum. The animals were bred in standard condition 12 hours: 12 hours dark and light cycle at temperature of around 25 degree Celsius. The animals were fasted for 48 hours prior to the experiment under standard laboratory conditions. After 48 hours, each group of rats received the

respective dose of test solution orally once daily for 7 consecutive days. The intraperitoneal injection of carbontetrachloride was performed 3 hours after the last extract administration on the 7th day except for group I, which received only 10% DMSO.

Experimental design: The mice were divided into seven different groups containing six mice in each group.

- Group-1: *Normal control:* The animals received 10% DMSO for 7 days.
- Group-2: *Acute toxicity control:* The animals received 10% DMSO 7 days and carbon tetrachloride/olive oil (50 % v/v, 0.5 ml/kg) given single dose [14].
- Group-3: Pretreatment with methanol extracts of organic tea at 200 mg/kg for 7 days followed by single dose of CCl4 on day 8.
- Group-4: Pretreatment with methanol extracts of non organic tea at 200 mg/kg for 7 days followed by single dose of CCl4 on day 8.
- Group-5: Pretreatment with methanol extracts of organic tea at 400 mg/kg for 7 days followed by single dose of CCl4 on day 8.
- Group-6: Pretreatment with methanol extracts of non organic at 400 mg/kg for 7 days followed by single dose of CCl4 on day 8.
- Group-7: *Positive control group:* Pretreatment with methanol extracts of Silymarin 25 mg/kg for 7 days followed by single dose of CCl4 on day 8.

Mice were dissected after ether anaesthesia and about 1 ml of blood was collected from hepatic portal vein and cardiac puncture. The liver from the dissected animal was isolated, washed with normal saline and preserved in 10 % formalin solution for histopathological studies. The blood was taken in test tube and was allowed to clot. The serum was separated and the serum concentration of Alanine transaminases (ALT), Aspartate transaminases (AST) and alkaline phosphatase (ALP) was determined by standard method using an autoanalyzer. The preserved and fixed liver was studied further with grossing and the tissue processing was carried out. Furthermore, the staining was performed using haematoxylin and eosin [15].

Statistical Analysis: The results of hepatoprotective activity were expressed as Mean ± S.E.M. Data analysis was done by using Microsoft Excel 2010 and Statistical Package for Social Sciences, Version 16.0 (SPSS V.16.0). Mean was compared by one way Analysis of variance (one way ANOVA) followed by post hoc Tukey's analysis for control, standard and test group comparisons. P values less than 0.05 is considered as significant.

Results

Hepatoprotective activity:

1. *Biochemical analysis:* The single dose of carbon tetrachloride/olive oil (50 % v/v, 0.5 ml/kg) was administered i.p. except normal control before 24 hours of dissection. CCl_4 treated mice results in the increment of liver enzymes like ALT, AST and ALP. The activity of ALT is 181.43±12.62 IU/L, AST is 115.48±15.07 IU/L and ALP is 276.8±18.73 IU/L. It was significantly higher than comparison to normal control group for which ALT is 32.68±1.81 IU/L , AST is 38.23±2.27 IU/L and ALP is 63.75±2.58 IU/L as listed in the table below. The levels of ALT, AST and ALP are represented in below figure respectively.

Fig-1: Bar diagram representing mean AST value of the different treatment groups. Results are expressed as Mean ± SEM. Indicates P value less than 0.005 as compared to normal control group (Group I). *Indicates P value less than 0.005 when compared to Control group (Group II), (Highly significant)

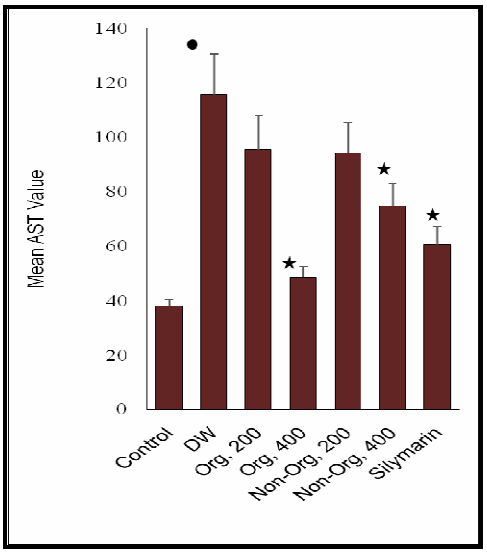


Fig-2: Bar diagram representing mean ALP value of the different treatment groups. Results are expressed as Mean ± SEM. Indicates P value less than 0.005 as compared to normal control group (Group I). *Indicates P value less than 0.005 when compared to Control group (Group II), (Highly significant)

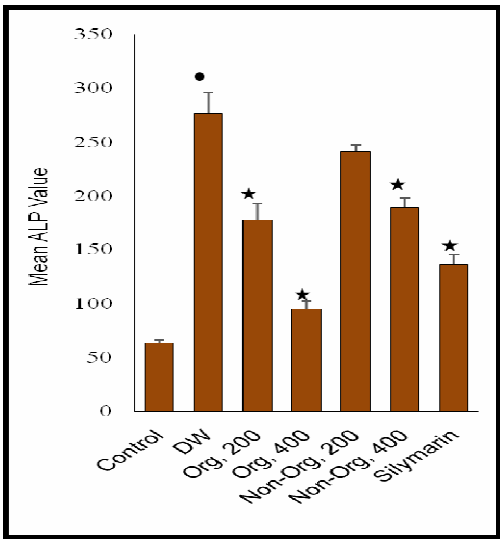
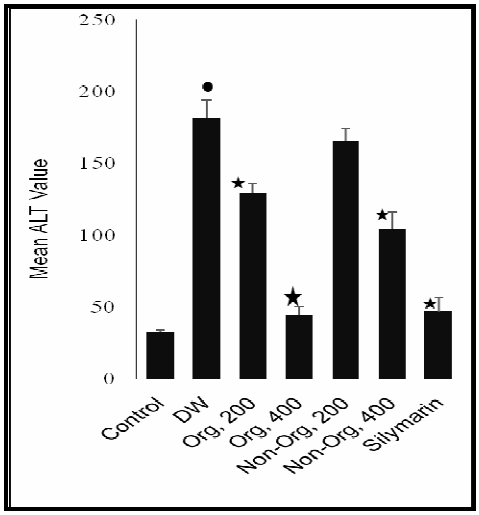


Fig-3: Bar diagram representing mean ALT value of the different treatment groups. Results are expressed as Mean ± SEM. Indicates P value less than 0.005 as compared to normal control group (Group I). *Indicates P value less than 0.005 when compared to Control group (Group II), (Highly significant)



2. *Histocytopathlogical analysis:* The observations seen in the histocytopathological study of the liver is shown in the table 1. It was observed that the mice injected with CCl4 have ballooning degeneration with confluent necrosis along with mixed infiltration which represents that the liver is highly injured. In case of methanol extracts of tea, the confluent necrosis has been treated but there is massive microvesicular steatosis in the mice treated with non organic extracts. Focal ballooning is seen in organic treated mice but it is not highly significant as it is traditional. The liver toxin produced by CCl_4 has been reversed by the Silymarin, which is the positive control of the experiment. All the features of the histological parameters are shown by the representative pictures photographed during the study as given below (Fig-4 to 7).

Table-1: Histopathological analysis

Group	CCl_4 toxic	Treatment	Ballooning degeneration	Cytic necrosis	Confluent necrosis	Submassive necrosis	Micro Vesicular steatosis	Macro Vesicular Steatosis	L^+ and mixed IF
1	_	_	normal	_	_	_	_	_	_
2	+	_	+	_	++	_	_	_	Mixed IF
3	+	Organic tea	focal	_	_	_	_	_	Focal L^+
4	+	Non organic tea	_	_	_	_	Massive	_	Focal L^+
5	+	Silymarin	normal	_	_	_	_	_	Patchy peri-venular

Fig-4: CCl4 induced hepatotoxicity

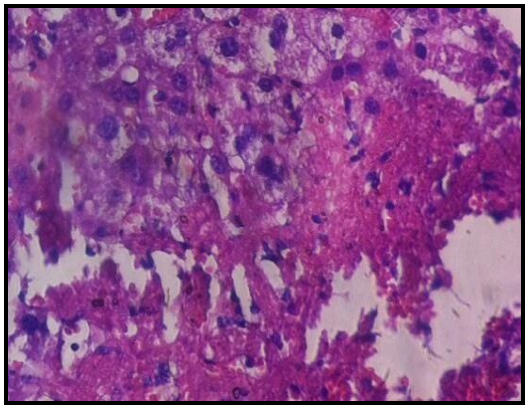


Fig-5: Organic treated mice liver histology

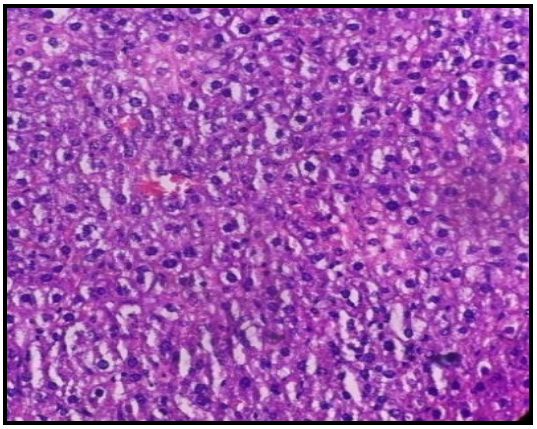


Fig-6: Inorganic treated mice liver histology

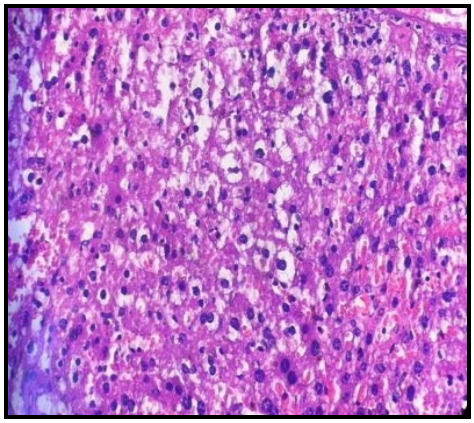
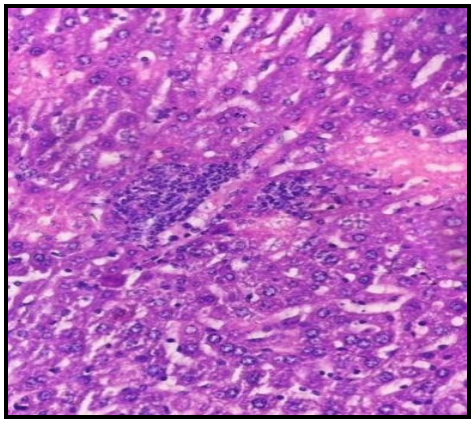


Fig-7: Silymarin treated mice liver histology



Discussion

Most of the diseases are caused by the oxidative stress. Free radicals which have one or more unpaired electrons are produced as a result of metabolism in normal or pathological cell which cause cell injury and wide range of diseases. Antioxidant compounds inhibit prevalence of diseases by inhibiting the oxidation of oxidizable materials through free radical scavenging and by diminishing oxidative stress [16].

CCl_4 is a toxic agent for liver that cause hepatic necrosis. The metabolites trichloromethyl radical and trichloromethly peroxyl radical from metabolism of CCl4 cause peroxidation and cause liver injury [17].Serum concentrations of Aspartate aminotransferase (AST) or glutamate oxaloacetate transaminase (SGOT) and Alanine aminotransferase (ALT) or glutamate pyruvate transaminase (SGPT) are the most commonly used biochemical markers of hepatic injury. These serum activities presumably increase as a result of cellular membrane damage and leakage [18]. Decreased levels of antioxidative enzymes, increased lipid peroxidation products and increase in the liver enzymes like ALT, AST and ALP along with hepatocellular necrosis occur, if CCl_4 is administered to the mice [19].

In the experiment the mice treated with CCl4 reports a tremendous increase in the enzymes level (ALT = 181.43 ± 15.07 IU/L, AST= 115.48±15.07IU/L and ALP= 276.8 ± 18.73 IU/L) as compared to normal controls. This indicate that there is severe hepatic damage due to CCl_4.The lipid peroxidation mediated necrosis of the cells might be one of the factors that not only cause damage to cell membrane increasing cytosolic ALT but also the mitochondrial membrane damage which increases the AST as well. Treatment with *Camelia sinensis* tea extracts lowers the levels of liver enzymes like ALT, AST and ALP. It represents, there is hepatoprotective action in

Camelia sinensis leaves. On a comparative basis, the organic tea showed better hepatoprotective action than that of non organic tea. Previous study showed that TFPS present in the flower of *Camelia sinensis* contained 3 polysaccharide fractions which is a natural polymer with antioxidant, hepatoprotective and antitumor activities [20].

Antioxidant activity of *Camelia sinensis* is responsible for the hepatoprotective activity. Based on the results of histopathology, the mice injected with CCl_4 followed by no any treating agents results in the necrosis of liver cells with focal ballooning degeneration and mixed IF. In case of mice treated with methanol extracts of organic tea, there is absence of necrosis but lymphocyte cells are observed representing acute injury. However, the mice treated with methanol extracts of non organic tea showed massive microvascular steatosis along with focal lymphocytes. Mice treated with Silymarin represents, a drug reverse the toxic action of CCl_4 characterized by the presence of patchy perivenular spots in mice. Thus, we can conclude that the hepatoprotective action of the organic tea has better action than non organic tea.

Conclusion

Organic tea is preferred over non-organic tea in respect of human consumption all over the globe. In Nepal, ethnomedicine suggests use of both kind of tea (Organic, Non-organic) as-functional food, potential source of antioxidants, treatment of various hepatic disorders, preventive care for chronic liver disease etc. Thus, with this scientific experimental study, organic *Camellia sinensis* is preferred oven non organic *Camellia sinensis* as hepatoprotective agent for numerous hepatic disorders.

Financial Support and sponsorship: Nil

Conflicts of interest: There are no conflicts of interest.

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Cite this article as: Khadka D, Aryal P, Parajuli DR, Shah A, Gautam N, Dhakal S and Adhikari BK. Evaluation of comparative hepatoprotective activity of organic versus non-organic *Camelia sinensis* methanol extracts in albino mice. *Al Ameen J Med Sci* 2019; 12(3):127-133.

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