



**AN ASSESSMENT OF THE CURATIVE IMPACT OF A POLYHERBAL REMEDY JALIN FOR CYCLOPHOSPHAMIDE-INDUCED TOXICITY IN MALE ALBINO RATS' LIVER**

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**ABSTRACT**

This study aimed to evaluate cyclophosphamide induced hepatotoxicity ameliorated by Jalinherbal extract (Ginseng, Lepediummeyerii, Liriosmaovata and Honey). Fifteen (15) albino male rats were grouped into three (3) and treated with cyclophosphamide and Jalin extract. Group one served as control and received distilled water, group two received 100mg/kg of cyclophosphamide per bodyweight of albino rats and group three received cyclophosphamide 24 hours before the administration of 2ml/mg of Jalin extract per bodyweight of albino rats. Blood samples were collected and analyzed for body weight, liver weight, liver to body weight ratio and liver enzymes (AST, ALT and ALP). Results showed a significant ( $P < 0.005$ ) increase in liver enzymes (ALT, AST and ALP). ALT increased from  $(32.20 \pm 1.64)$  to  $(96.60 \pm 1.02)$ , AST increased from  $(52.60 \pm 1.52)$  to  $(110.70 \pm 1.17)$  while ALP  $(224.23 \pm 2.30)$  to  $(260.20 \pm 2.40)$  when compared to control. On the other hand, a significant decrease was observed in the level of serum AST, ALP and ALT following treatment with jalin extract and cyclophosphamide when compared to the cyclophosphamide group only. ALT decreased from  $(96.60 \pm 1.20)$  to  $(52.60 \pm 2.90)$ ; AST decreased from  $(110.70 \pm 1.17)$  to  $(67.20 \pm 1.30)$  while ALP decreased from  $260.20 \pm 2.40$  to  $(235.40 \pm 3.90)$  after treatment with jalin extract and cyclophosphamide. Histopathology of the rat liver treated with cyclophosphamide alone showed degeneration of the central vein, necrosis, occluded sinusoidal space and ballooning of hepatocytes. Rats treated with Jalin extract and cyclophosphamide showed normal morphology of the liver, central vein, hepatocytes with intact sinusoidal space similar to the histopathology of control rats. Conclusively, jalin extract is a curative potential herbal remedy against liver dysfunction and should be encouraged.

**KEYWORD:** Cyclophosphamide, Jalin extract, Toxicity, Liver.

**INTRODUCTION**

Cyclophosphamide is an effective chemotherapy drug used to treat cancer.<sup>[20]</sup> It is an alkylating substance that creates a bond between two DNA strands, causing the connection to break and impeding the creation of RNA and protein as a result.<sup>[18]</sup> A larger dose of the medication typically results in a reduced host defense mechanism despite the treatment having a suitable anticancer effect and reducing tumor growth.<sup>[9]</sup> This weakness usually results in the suppression of immune-logical responses, the development of opportunistic infections, and the relapse of cancer.<sup>[20]</sup> Cancer animals treated with modest doses of cyclophosphamide experience increased spleen lymphocyte proliferation, whereas pre-treatment proliferation is decreased.<sup>[9]</sup> The GI tract effectively absorbs cyclophosphamide, which is then broadly dispersed throughout bodily tissues.<sup>[20]</sup> This medication is metabolized by the liver and excreted through the urine<sup>[1]</sup>

One of the most essential organs for removing toxins from the body is the liver, which must continually deal with many poisons and metabolites.<sup>[4]</sup> Problems with the liver can also be numerous and diverse.<sup>[26]</sup> Hepatocytes use smooth endoplasmic reticulum enzymes, oxidation, and methylation to deactivate toxic substances.<sup>[3]</sup> Similar to other anticancer medications, cyclophosphamide damages the liver and produces cytotoxicity in rapidly proliferating cells.<sup>[20]</sup> The etiology of cyclophosphamide toxicity in liver cells is unknown, however it appears that enhanced free oxygen radicals produced by cyclophosphamide metabolites play a significant part in its development, according to.<sup>[1]</sup> A few enzymes and metabolic pathway byproducts that could be used as biochemical indicators of liver dysfunction include serum bilirubin, plasma proteins, alanine amino transferase, aspartate amino transferase, ratio of aminotransferases, alkaline phosphatase, gamma glutamyltransferase, 5' nucleotidase, ceruloplasmin, and

–fetoprotein.<sup>[22]</sup> The liver is primarily responsible for manufacturing the majority of the proteins that are released into the blood, including apolipoprotein, carrier proteins, hormones, prohormones, and hemostasis and fibrinolysis factors.<sup>[3][16]</sup>

Nevertheless, it has been noted that a variety of medicinal herbs are essential for the detoxification of hazardous chemicals in both human and animal models.<sup>[23]</sup> Approximately 80% of the world's 5.2 billion people rely solely on medicinal plants for their healthcare needs.<sup>[15]</sup> Some chemically active components of these plants known as secondary metabolites are what give them their therapeutic significance.<sup>[27]</sup> These phytochemicals or secondary metabolites have important pharmacological effects, including anti-oxidative, anti-allergic, antimicrobial, hypoglycemic, and anti-carcinogenic qualities.<sup>[6]</sup> It has been demonstrated that naturally existing plants and plant products combined into certain recipes may interact. These outcomes include mutual augmentation, mutual aid, mutual constraint, and mutual hostility, according to.<sup>[12]</sup> Polyherbal compounds are used to treat a variety of infections (Chickenpox, Common Cold, Diphtheria, E. coli, Giardiasis, Infectious Mononucleosis, Influenza), as well as to treat and manage metabolic and neurodegenerative diseases, due to cultural and socioeconomic factors in Africa, most notably the Nigerian system of medicine.<sup>[2][12]</sup> Natural This can occasionally result from using synthetic medications<sup>[5]</sup>

One common and popular polyherbal used in Nigeria is Jalin Herbal Mannex Liquid with Nafdac No A7-2077L, it is a polyherbal formulation used to enhance sexual performance in men, fight weak erection, overcome impotence, improvement of sperm count, eliminate waist pain, increase libido and enhance longer sex for the satisfaction of both partners.<sup>[5]</sup> It contains honey, *Panax ginseng*, *Lepidium mehenil* and *Liriosma ovate*.

Ginseng, maca, and honey are the three main components of genuine Jalin extract, a therapeutic extract. Since ancient times, ginseng has been a widely used herbal remedy and a key ingredient in many pharmaceutical medications.<sup>[21]</sup> Ginnosides and gintonin are substances that define ginseng. Numerous physiological effects of ginseng, including those on the circulatory, immunological, and neurological systems, have been documented.<sup>[19]</sup> It has been utilized to improve sex performance and fulfillment.<sup>[21]</sup>

Maca comprises a number of secondary metabolites, including glucosinolates, alkaloids, macamides, and macaridine.<sup>[24]</sup> Benzyl glucosinolate (glucotropaeolin) and m-methoxybenzylglucosinolate are the two aromatic glucosinolates found in the highest concentrations in Maca.<sup>[25]</sup>

Natural honey is a sweet, viscous fluid produced by honeybees from plant nectar, plant secretions, or insect

excretions that have been fed on live plants.<sup>[22]</sup> Honeybees collect these substances, transform them, mix them with other substances, then store and allow them to ripen and mature in the honey comb.<sup>[22]</sup> Honey is one of nature's wonders.<sup>[14]</sup> Its antibacterial, anti-inflammatory, and anti-oxidant properties, as well as the strengthening of the immune system, have been endorsed for their positive effects.<sup>[14]</sup> Given the foregoing context, the aim of this study was to examine the therapeutic potential of jalin extract in cyclophosphamide-induced liver damage in male albino rats.

## MATERIALS AND METHODS

### Chemicals

All chemicals used in this study were of analytical reagent grade.

### Purchase of Jalin Herbal Mannex Liquid

The Jalin Herbal Mannex Liquid (MFG 8 2020 ExP 8 2022) batch number BNO: 320. With Nafdac registration number A7-2077L was purchased from Cynflac Pharmacy Limited, Yenegoa, Bayelsa State.

### Experimental Animals

From the Niger Delta University's animal home on Wilberforce Island in Bayelsa state, fifteen (15) healthy adult male wister rats weighing between 120g and 260g were taken. For a period of six weeks, they were fed with regular feed (pellet) and purified water. In order to manage and monitor experiments on animals, all protocols were carried out in compliance with the Institutional Animal Ethical Committee's (IAEC) guidelines.

### Experimental Design

A total of fifteen (15) adult albino rats grouped into three (3) groups each having five rats

**Group 1:** received distilled water and standard feed for 6 weeks

**Group 2 (Positive control):** received 100mg/kg body weight (bw) of Cyclophosphamide (given once via intraperitoneal injection) and distilled water after 24 hours for 6 weeks.

**Group 3 (Test group):** received 2ml/kg per body weight of jalin herbal mannex liquid after 24 hours of administration of cyclophosphamide for 6 weeks.

The administration of cyclophosphamide was done 24 hours before the administration of Jalin herbal mannex liquid. 24 hours after the last administration, the rats were dissected and blood sample was collected. The liver was also harvested and processed for biochemical analysis. The blood was centrifuged and the serum taken and stored in the refrigerator. Part of the liver was preserved with formalin for histopathological studies, while the other part was mixed phosphate buffer and homogenised.

### Sample Collection

#### Liver

After 6 weeks of oral administration of jalin herbal mannex liquid the rats were slaughtered using chloroform anaesthetization. The liver was obtained, washed in normal saline, weighed, homogenised using phosphate buffer (PH 7.4) and centrifuged for 10 minutes at 2000rpm and their serum was collected. The serum was kept in sample bottles containing formalin and was subjected to histological analysis.

#### Biochemical Assay

According to the Reitman and Frankel<sup>[22]</sup> principle, the activities of serum Aspartate transaminase (AST) and Alanine transaminase (ALT) were measured in total. Activities expressed as U/L. Alkaline Phosphatase was assayed according to the method of Rec.<sup>[21]</sup> Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by the colorimetric technique using a commercial assay kit from Randox Laboratories

Ltd, Co. Antrim, United Kingdom, in order to assess hepatic derangement in the animals utilized for the study. Using Randox Laboratories Ltd. test kits, the colorimetric approach was used to estimate alkaline phosphatase (ALP).

#### Statistical Analysis

All data obtained were presented as mean and standard deviation (Mean  $\pm$ SD). The SPSS Software of version 23.0 was used for the analysis of the data obtained. Comparison of result between control and test was done using one-way analysis of variance (ANOVA and group means were compared using Bonferroni multiple comparison. Level of significance was determined at a probability level of  $p < 0.05$ .

#### RESULTS

The results for the effect of Jalin herbal mannex liquid on liver of male wistar albino rats is presented in Tables 3.1 and 3.2.

**Table 3.1: The effect of administration of cyclophosphamide and the Jalin extract on the body weight and liver weight of albino rats.**

Treatment	Body weight (initial) (g)	Body weight (final) (g)	Liver weight (g)	Relative liver weight (g)
Control (distilled water)	170.70 $\pm$ 8.15 <sup>a</sup>	199.6 $\pm$ 10.03 <sup>a</sup>	9.88 $\pm$ 1.40 <sup>a</sup>	4.9 1 $\pm$ 1.71 <sup>a</sup>
Cyclophosphamide alone	195.10 $\pm$ 8.04 <sup>b</sup>	215.20 $\pm$ 10.50 <sup>b</sup>	6.80 $\pm$ 1.23 <sup>b</sup>	3.16 $\pm$ 1.00 <sup>b</sup>
Cyclophosphamide + jalin herbal extract	171.30 $\pm$ 75 <sup>c</sup>	191.3 $\pm$ 9.87 <sup>c</sup>	8.10 $\pm$ 2.11 <sup>c</sup>	4.24 $\pm$ 1.30 <sup>c</sup>

Data are mean  $\pm$ SD (n=5). Mean in the column with different superscript letter(s) are significant different, ( $P < 0.05$ ) one-way ANOVA.

There was significant decrease ( $P < 0.05$ ) in the weight respectively in albino rats treated with cyclophosphamide (100mg/kg body weight) compared to control respectively. There was significant increase

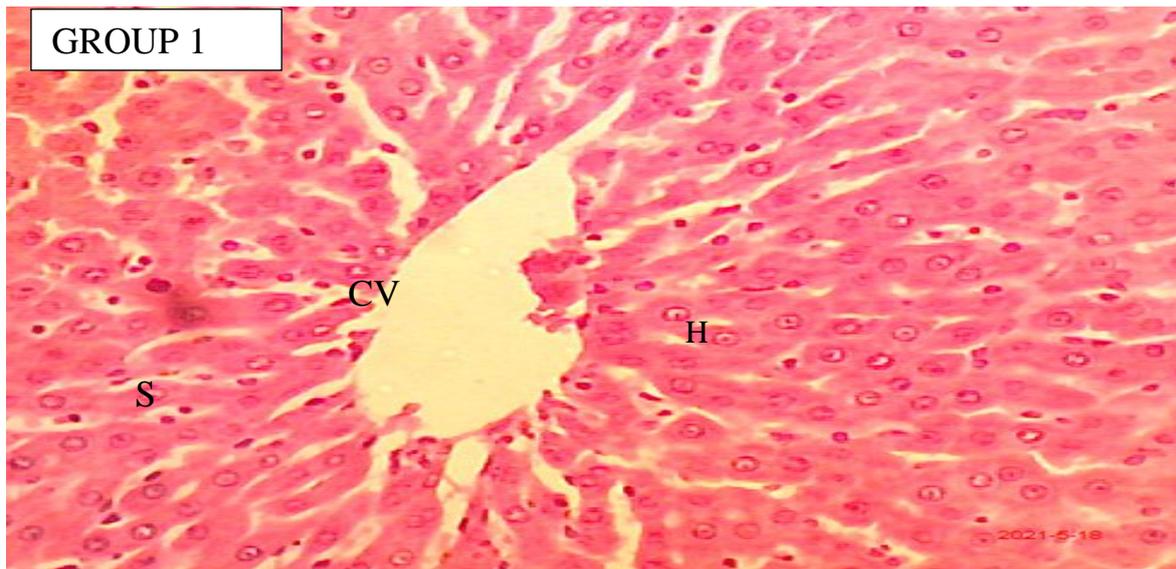
( $P < 0.05$ ) in the weight of albino rats treated with cyclophosphamide and jalin extract compared to positive control (group with cyclophosphamide alone).

**Table 3.2: Effect of administration of cyclophosphamide and Jalin extract in serum AST, ALT and ALP of albino male rats.**

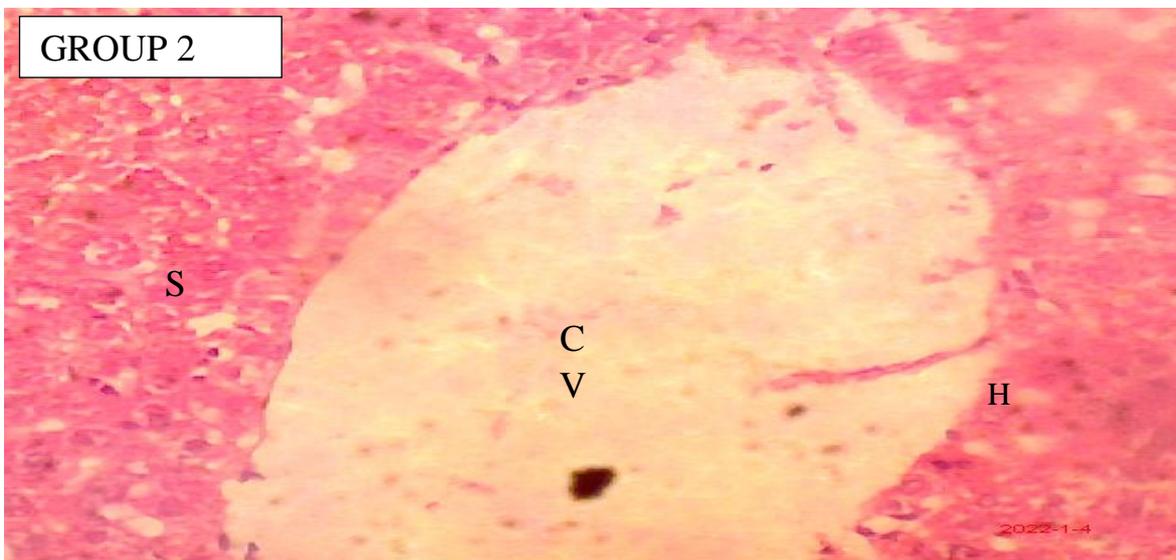
Treatment	AST( U/L)	ALT (U/L)	ALP (U/L)
Control(distilled water)	52.60 $\pm$ 1.52 <sup>a</sup>	32.20 $\pm$ 1.64 <sup>a</sup>	224.23 $\pm$ 2.30 <sup>a</sup>
Cyclophosphamide alone	110.70 $\pm$ 1.17 <sup>b</sup>	96.60 $\pm$ 1.02 <sup>b</sup>	260.20 $\pm$ 2.40 <sup>b</sup>
Cyclophosphamide +Jaliñ extract	67.20 $\pm$ 1.30 <sup>c</sup>	52.60 $\pm$ 2.90 <sup>c</sup>	235.40 $\pm$ 3.90 <sup>c</sup>

Data are mean  $\pm$  SD (n=5). Mean in the same column with different superscript letter (s) are significantly different, ( $P < 0.05$ ).

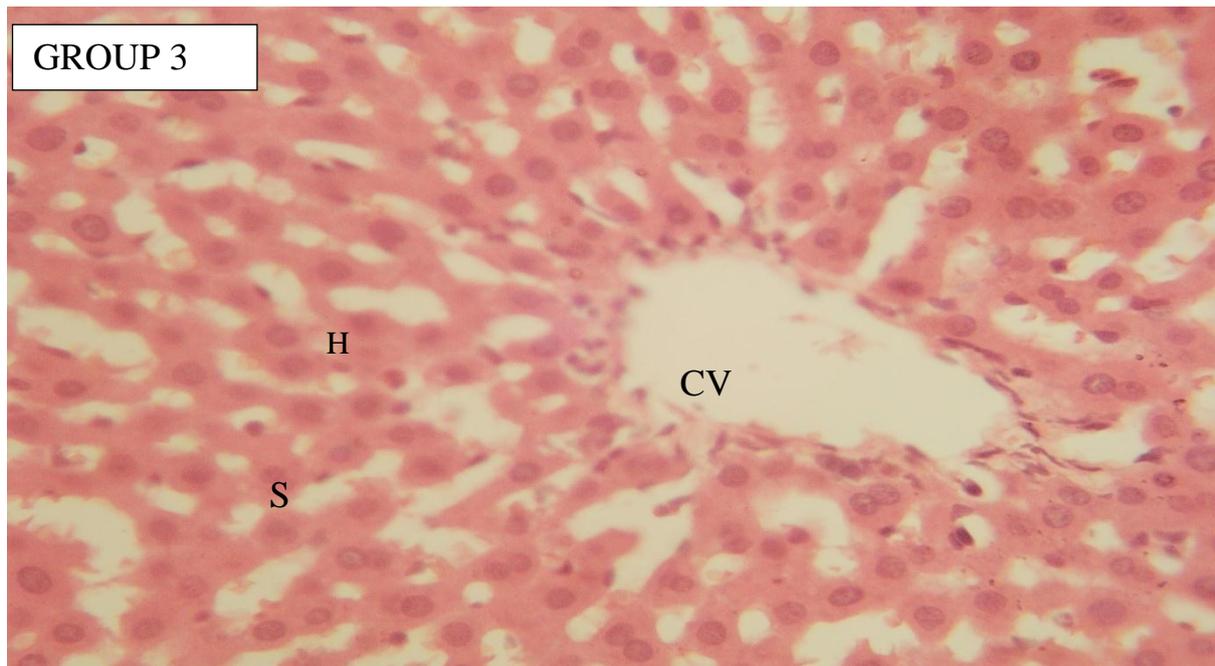
There was significant increase ( $P < 0.05$ ) in serum AST, ALP and ALP respectively in male albino rats treated with cyclophosphamide (100mg/kg per body weight) compared to the control group respectively. On the other hand, there was significant decrease ( $P < 0.05$ ) in serum AST, ALT and ALP in albino male rats treated with cyclophosphamide and Jalin extract when compared to control.



**Fig1:** Shows the morphology of the normal liver. Slide shows normal morphology of the liver, central vein (CV), hepatocytes (H) with intact sinusoidal space (S) (X40)H&E.



**Fig2:** Shows the morphology of the liver after the administration of 100mg/kg body weight (bw) of Cyclophosphamide. The slide shows degeneration of the central vein (CV) with areas of necrosis, occluded sinusoidal space (S) and ballooning of hepatocytes(H)(X40)H&E.



**Figure 3:** Shows the morphology of the liver after the administration of 2ml/kg per body weight of Jalin herbal mannex liquid and 100mg/kg body weight (bw) of Cyclophosphamide. Slide shows normal morphology of the liver, central vein (CV), hepatocytes (H) with intact sinusoidal space (S) (X40)H&E.

#### DISCUSSION

Cyclophosphamide, a common anticancer drug, exhibits a wide range of antitumor efficacy.<sup>[20]</sup> The recovery of these alterations, however, depends on the type and cumulative dose in addition to the time of exposure, and the duration of usage of an anticancer treatment might result in certain adverse effects.<sup>[21]</sup>

In this study, treatment with cyclophosphamide decreased liver weight from  $9.88 \pm 1.40$  to  $6.80 \pm 1.23$  when compared to the control group. Treatment with cyclophosphamide (100mg/kg per body weight of albino) + Jalin extract (2ml/kg bw of albino rats) increased the body weight  $171.30 \pm 7.15$  to  $195.10 \pm 8.04$  when compared to the cyclophosphamide (alone) group (Table 3.1). Cyclophosphamide increased the activity of liver enzymes; ALT, AST and ALP from  $32.20 \pm 1.64$  to  $96.60 \pm 1.02$ ,  $52.60 \pm 1.52$  to  $110.70 \pm 1.17$ ; and  $224.23 \pm 3.40$  to  $260.20 \pm 2.40$  respectively when compared with the control group. In a related study,<sup>[1]</sup> found that the administration of cyclophosphamide caused hepatocyte necrosis and marked rises in the levels of several enzymes, including alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, gamma, glutamyltransferase, total bilirubin and conjugated bilirubin. The importance of medicinal plants cannot be overlooked, since most herbal plants are used for sustaining health and alleviating or treating health issues.<sup>[7]</sup> Some of the herbal plants have been certified as drug and are claimed to be safe and effective.<sup>[8]</sup> In recent years, much attention has focused on this plant for its potential effects on sexual function.<sup>[13][21]</sup>

In this study, the treatment of the herbal extract (Jalin) and cyclophosphamide decreased the activity of the liver enzymes ALT, AST and ALP from  $96.60 \pm 1.02$  to  $52.60 \pm 2.90$ ,  $110.70 \pm 1.17$  to  $67.20 \pm 1.30$  and  $260.20 \pm 2.40$  to  $225.40 \pm 3.90$  respectively in the serum of albino rats compared to the ground treated with cyclophosphamide alone (Table 3.2). This result is consistent with the findings of<sup>[10]</sup> which showed that the administration of ginseng hydro-alcoholic extract ameliorated cyclophosphamide induced liver damage. Also, similar findings were observed in a study conducted by<sup>[17]</sup> which showed the hepatoprotective effect of gallic acid in cyclophosphamide-induced hepatic damage indicated by significant elevation liver enzymes in rats.

In this study, photographs of rat liver treated with cyclophosphamide showed degeneration of the central vein with areas of necrosis, occluded sinusoidal space and ballooning of hepatocytes (Fig 2). Rats treated with Jalin herbal mannex liquid and cyclophosphamide showed normal morphology of the liver, central vein, hepatocytes with intact sinusoidal space (Fig 3) similar to the histopathology report of control rats (Fig 1). This finding showed that Jalin Herbal Mannex liquid treatment on the rats had a positive effect on the liver. This is in line with the findings of<sup>[10]</sup> where liver histopathology findings show direct and obvious toxic effects of cyclophosphamide ameliorated by hydroalcoholic extract of ginseng.

Furthermore, the treatment with Jalin herbal extract did not cause significant changes in the parameters compared to the treatment with cyclophosphamide alone which caused significant changes in the parameters. While on the other hand, the treatment with cyclophosphamide and

Jalin herbal extract maintained the levels of all the parameters closer to control group. The elevation of liver enzymes (AST, ALT and ALP) in this study could be due to hepatic injury associated with the administration of cyclophosphamide. The reduction in liver enzymes activities observed following the administration of jalin extract and cyclophosphamide showed that the administration of jalin extract have hepatoprotective effect.

## CONCLUSION

This study shown that Jalin extract has curative and preventive effects on CP-induced liver toxicity. Jalin extract may therefore have a variety of protective benefits against liver problems, making its use recommended.

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