

JOPAT Vol 20(1) 581– 597, Jan – June. 2021Edition

ISSN2636 – 5448 <https://dx.doi.org/10.4314/jopat.v20i1.4>**The ameliorative effect of the solvent extracts of *Ocimum basilicum* against acetaminophen-induced liver damage in albino rats**Titilayo. M. Asala¹, Mathew. O. Abatan², *Suliat. A. Salami¹, Oluwatosin. O. Baba-Onoja², Olatunde. B. Akanbi³, Adekunle. B. Rowaiye¹, Benjamin. O. Ocheja⁴, and G. Ada⁵¹.Department of Medical Biotechnology, National Biotechnology Development Agency
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Nigeria³ Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ilorin, Nigeria⁴ Department of Physiology and Biochemistry, University of Benin, Benin City, Nigeria.⁵ Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria,
Nigeria**ABSTRACT**

This study was designed to evaluate the protective activities of 4 different *Ocimum basilicum* L whole plant extracts against acute acetaminophen-induced liver damage in albino rats. A total of 42 rats were divided into 7 groups comprising the control (acetaminophen, water, and silymarin treated) groups and OB-treated (chloroform, diethylether, ethylacetate, and methanol extract) groups. Each treatment group was made up of 6 rats, with 3 replicates of 2 rats each. At the end of the treatment period, blood samples were collected to investigate the activities of liver enzymes, and liver tissue was harvested for histological analysis. Rats pre-treated with OB extracts showed decreased ($p<0.05$) ALT, AST, and ALP activities when compared with the silymarin-treated and positive control groups. Serum urea decreased ($p<0.05$) in OB-treated groups compared to the control groups and least ($p<0.05$) activity was seen in rats pre-treated with diethyl ether and methanol extracts. Rats pre-treated with OB extract (except diethyl ether extract) showed decreased ($p<0.05$) creatinine activity in comparison with the positive control group. The decreased value of serum total bilirubin and D bilirubin in rats pretreated with OB methanol extract was comparable to those of negative control. Histopathological examination revealed hepatic tissue necrosis in the acetaminophen-induced rats and varying degrees of injury and amelioration in other groups. The results from of all the solvent extracts of OB whole plant suggest significant hepatoprotective and antioxidant activities with the methanolic extract having higher hepatoprotective activity than the standard, Silymarin, and other solvent extracts.

Keywords: *Ocimum basilicum* L., hepatoprotective activity, albino rats, acetaminophen.**Corresponding author*** Salami S. A., salami.suliata@pg.funaab.edu.ng, +2347087496840©2007

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INTRODUCTION

The liver is one of the largest organs in human body and the major site for metabolism and excretion [1]. It plays a vital role in maintaining metabolic homeostasis, biotransformation of chemicals compounds, detoxification, and excretion of many endogenous and exogenous chemical compounds in the body [2, 3]. Thus, maintaining a healthy liver is a crucial factor for overall wellbeing and any injury or impairment of its functions may result to many health implications [4].

Hepatic diseases are a global health challenge, and are triggered mainly by viruses, aflatoxins, water pollutants, metabolic diseases, alcohol, drugs, or chemical compounds [5-7]. Acute liver damage is characterized by hepatocellular necrosis, parenchymal inflammation, and varying degrees of zone 1, 2, and 3 pathologies of the hepatic tissue [8-9]. Hepatotoxicity is one of the main reasons behind withdrawal of a drug from the circulation. Fifty percent of all acute liver failures and 5% of all hospital admissions are associated with drug-induced hepatotoxicity [10].

Paracetamol or acetaminophen is the most common cause of acute liver damage in the United States because it is easily accessible as an over-the-counter medication [11].

Acetaminophen is widely used as an analgesic, and antipyretic drug and is well known to cause hepatotoxicity at overdose [11]. A significant amount of acetaminophen is metabolized by the cytochrome P450 system where it is directly conjugated with glucuronic acid or sulfate [12]. When the two conjugation pathways are over-saturated, a toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI) is formed. NAPQI forms adducts with the mitochondrial proteins involved in the electron transport chain ([13-14]. This leads to the generation of superoxide and highly reactive peroxynitrite species culminating in oxidative and nitrosative stress [15]. Therefore, though acetaminophen is safe at therapeutic doses, overdose causes mitochondrial dysfunction and centrilobular necrosis in the liver [12].

Drug-induced liver injury (DILI) which is largely due to acetaminophen overdose is the most prevalent cause of acute liver failure in the United States and also the most common reason for the withdrawal of medicines from the market [16]. Severe DILI, may result in need for transplantation and continuing treatment with a drug that has begun to cause liver injury may increase the risk of not only developing more severe acute damage but also chronic injury and death [17-19]. Rising cases of liver damage and diseases reported in Nigerian hospitals have been linked to

increase in alcohol consumption, Hepatitis B Virus cases, ingestion of herbs and roots and cigarette smoking [20-23]. Prior to exposure to paracetamol Patients with risk factors, are at risk of developing severe toxicity from paracetamol dosage that are considered safe [24].

In view of the growing concerns on drug induced liver injury, there is a need to prospect for safe and efficacious drugs that can protect the liver from damage, stimulate liver function, and help to regenerate hepatic cells [25]. As the world gravitates towards an increasing engagement of traditional medicine, the need to prospect for plant-based natural products with hepatoprotective activity becomes imperative [26]. Several medicinal preparations in Ayurveda have been recommended for the treatment of liver disorders [27]. One of such Ayurvedic medicines is Silymarin, which is extracted from milk thistle seeds. Silymarin contains flavonolignans and it exhibits profound cytoprotective activity. Silymarin shows anti-inflammatory properties through the TNF- α inhibition; antioxidant activity and membrane-stabilizing properties; inhibits apoptosis and fibrogenesis in the liver; and promotes hepatocyte regeneration. [28-29]. Studies have shown that the long-term administration of Silymarin, significantly increases survival time in patients with alcohol-induced liver cirrhosis [29].

Ocimum basilicum is an annual plant mostly found in the tropical, subtropical, and temperate regions of the world. A member of the labiatae (lamiaceae) family of plants, *Ocimum basilicum* contains wide variety of phytochemical constituents of medicinal value [30-31]. *Ocimum basilicum* is known to possess strong antioxidant and antimicrobial activities due to its phenolic acids and aromatic compounds [32]. These include volatile oils, saponins, coumarins, alkaloids, tannins, anthra-quinones, anthocyanins, flavonoides, diterpenoides, tri-terpenoides, pyredines, pyrolidines, polyphenols, irridoides, quinones, sugars, and insect moulting hormones [30]. This plant can also be used as carminative, stimulant, diaphoretic, diuretic, dyspepsia, antiseptic, anesthetic, anti-spasmodic, anthelmintic, anti-diarrheal, analgesic, anti-tussive, and antiemetic agents [31].

In the present study, we examined the effect of *O. basilicum* extracts on acetaminophen-induced hepatotoxicity and its antioxidant potential in albino rats. Silymarin was used as a reference compound because of its well established hepatoprotective and antioxidant activity. It is hoped that this project would culminate in the discovery and development of plant-based therapies to combat acute and chronic liver injuries.

MATERIALS AND METHODS

Drugs

The experiment was carried out at the Department of Biochemistry, National Veterinary Research Institute, Vom, and Plateau State, Nigeria.

Acetaminophen and Silymarin

The silymarin and acetaminophen used for this experiment were purchased from La Med Pharmacy Jos, Plateau state, Nigeria.

Plant Collection and Preparation of Extracts

Whole plant of *Ocimum basilicum* harvested from Molete Bode, Ibadan Nigeria was identified at the Department of Botany, Faculty of life Science, University of Ibadan. The plants were air dried and pulverized. Chloroform, diethylether, ethylacetate and methanol were used for solvent to solvent extraction of the homogenized dried matter of the plants of OB with percentage yields of 3.8%, 1.9%, 0.9% and 2.12% respectively.

Preliminary Phytochemical Screening of plant extract

Qualitative analysis of the phytochemical constituents of OB extracts was done to determine the presence or absence of some phytochemicals following the procedure of Evans and Yakubu [33]. The screening involved the detection of secondary metabolites such as Saponins, Tanins,

Steroids, Cardiac Glycosides, Anthraquinones, Flavonoids, Alkaloids, and Terpenes.

Experimental Animals

Forty-two albino rats of both sexes (weighing 200 ± 20 g) were sourced from the small animal experimental station of the National Veterinary Research Institute, Vom, Plateau State and acclimatized for two weeks at room temperature. They were housed in the animal house of National Veterinary Research Institute, Vom Plateau State, and were administered with a commercial animal feed and water *ad libitum* throughout the experiment. The housing and handling of animals were in line with ethical standards.

Acute toxicity study

The limit test for acute toxicity was performed with five rats following the up and down procedure of OECD (34) The aqueous extract of OB was administered at a dose of 3000mg/kg. No mortality was observed 14 days after post treatment.

Experimental design

A total of forty-two albino rats were grouped into seven groups of six animals each. Each treatment group was made up of 6 rats, with 3 replicates of 2 rats each. The animals were divided into 3 control groups (positive,

negative, and standard) and 4 treatment groups receiving the various solvent extracts.

I: Positive control group – received acetaminophen for seven days at 750mg/kg per os and distilled water day 8.

II: Negative control group - received only feed and water.

III: Standard control group– pre-treated with Silymarin (100mg/kg) for seven days and was administered with acetaminophen (750mg/kg) per os on day 8

IV – pre-treated with 1200mg/kg of chloroform extract of OB for seven days and was administered with acetaminophen (750mg/kg) per os on day8

V- pre-treated with 1000mg/kg of ethyl acetate extract of OB for 7days and was administered with acetaminophen (750mg/kg) per os on day 8.

VI- pre-treated with 1200mg/kg of diethyl ether extract of OB for 7 days and was administered with acetaminophen (750mg/kg) per os on day 8

VII- pre-treated with 1000mg/kg of methanolic extract of OB for 7 days and was administered with acetaminophen (750mg/kg) per os on day 8.

Serum biochemical analysis

On the 9th day of the study, 2mls of blood each were collected from forty-two albino rats by cervical venipuncture into sample bottles

for serum biochemical analyses. Blood samples without anticoagulant were placed in a Centrifuge 800D (Peacesky, Germany) and centrifuged at 3,000 rpm for 10 minutes to obtain the serum. The serum was stored at 4°C. The activities of alkaline phosphatase (ALP), total bilirubin, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and blood urea nitrogen (BUN) were determined according to the methods described by Reitman and Frankel [35]. Commercial Randox kits (Randox laboratories UK) were used for all serum metabolite analyses except Creatine kinase MB which was assayed with the aid of kit obtained from Biosystems reagents and Instruments, (Spain). Vis Spectrophotometer (721 (D), PEC Medical, USA) was used to carry out these analyses.

Histopathology

On the 9th day of the study, the animals were slaughtered after their weights were taken. The heart, stomach kidney, spleen, and liver of the rats were harvested and weighed. The percentage weights of the harvested organs relative to the carcass were determined. Small pieces of liver tissues were collected in 10% formaline buffer for proper fixation. The tissues were embedded in paraffin wax, histological sections of 5–6 µm in thickness

were made and stained with hematoxylin and eosin for examination [36].

Data analysis

The value was analysed as Means \pm SEM and analyzed by One-way analysis of variance (ANOVA), followed by the Tukey post-hoc test for multiple comparison using Graph pad prism5 adapted, $P \leq 0.05$ were considered statistically significant.

Ethics approval

“All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. In line with the ethics regulations stated in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health [37]. The research group ensured all the rats received humane care throughout the period of the study

RESULTS

Phytochemicals detected in extract: Results on Table 1 reveal the phytochemicals detected in the different extracts of OB. The analysis revealed the presence of saponins, tannins, steroids, cardiac glycosides, flavonoids, and terpenes in chloroform, ethylacetate, diethylether, and methanol extracts of OB. Saponin was detected only in the ethylacetate extract. Cardiac glycosides, flavonoids, and tannins were detected in all the extracts.

Steroid and terpenes were detected in all extracts except chloroform. Alkaloids and anthraquinones were not detected in all the solvent extracts.

Serum Biochemistry Parameters: The effects of silymarin, chloroform, ethylacetate, diethylether, and methanol extract of *O. basilicum* on serum chemistry of rats with damaged liver were determined and results presented on Table 2. It was observed that rats pre-treated with OB extracts had decreased ($p < 0.05$) ALT, AST, and ALP when compared to the silymarin-treated and the positive control groups. However, there was increase ($p < 0.05$) in activity of ALT when compared with the negative control. The activity of ALT seen in negative control group was similar to those seen amongst rats pre-treated with methanol extract of OB. Amongst the OB treated groups, rats pre-treated with methanol extract, ethyl acetate, and chloroform extracts had the least ($p < 0.05$) ALT, AST and ALP activities, respectively.

Serum urea decreased ($p < 0.05$) in OB-treated groups compared to the control groups and least ($p < 0.05$) activity was seen in rats pre-treated with diethyl ether and methanol extracts. Rats pre-treated with OB extract (except diethyl ether extract) showed decreased ($p < 0.05$) in creatinine activity in comparison with the positive control group.

However, when compared with the negative control and silymarin-treated group, an increase ($p < 0.05$) in activity was seen in rats pre-treated with all OB extract except chloroform extract which had the overall least creatinine activity. Total bilirubin and D bilirubin in rats pre-treated with OB extracts (except methanol) was increased ($p < 0.05$) when compared with the control groups. The decreased value of serum total bilirubin and D bilirubin in rats pre-treated with OB methanol extract was comparable to those of negative control.

Serum total protein in rats pre-treated with OB extracts decreased when compared with the positive and negative control groups. However, serum total protein increased ($p < 0.05$) in rats pre-treated with OB extract (except chloroform extract) when compared with the silymarin-treated group. There were no differences ($p > 0.05$) in the serum albumin of control groups and OB-treated group. However, the concentration seen in rats pre-treated with chloroform extract was decreased ($p < 0.05$) in comparison with the control groups and other OB-treated groups.

Histopathology analysis: Figures 1 (A-H) show the histopathology results of the liver section treated with acetaminophen and various concentrations of OB solvent extracts.

The liver samples of rats used for acute toxicity (administered 3000 mg/kg OB solvent extract) revealed severe diffuse hepatocytes individualization, hyper-eosinophilic cytoplasm, and nuclei karyorrhexis (hepatocellular necrosis) (Figure 1A). Centrilobular and hepatic necrosis was observed in the liver section of rats in the positive control group (Acetaminophen) (Figure 1B). Rats pre-treated with silymarin showed severe diffuse centrilobular and midzonal hepatocellular necrosis often times bridging with severe diffuse haemorrhage (Figure 1D). Moderate diffuse hepatocellular nuclei pyknosis was observed in the liver of rats pre-treated with chloroform extract (Figure 1E). Rats pre-treated with ethylacetate extract showed diffuse centrilobular and midzonal hepatocellular necrosis, severe vascular congestion, and hemorrhage in the liver (Figure 1F). For rats pre-treated with ether extract, severe diffuse hepatocellular necrosis, with occasional apoptotic bodies was observed in the liver (Figure 1G). Rats pre-treated with methanolic extract, showed moderate histiocytosis (neutrophilic infiltration) in the liver (Figure 1H), while in the kidney there was diffuse acute tubular coagulative necrosis and glomerular necrosis.

DISCUSSION

The qualitative test done to determine the phytochemical constituents of OB solvent

extracts revealed the presence of cardiac glycosides, flavonoids, tannins, steroids, terpenes, and saponins and thus corroborates the reports of earlier studies [30-31]. This observation suggests that OB whole plant have free radical scavenging and antioxidant properties as reported by Osadebe et al. [38]. The presence of the flavonoids, tannins and saponins may contribute to the hepatoprotective activity of the OB extract

Rats pre-treated with OB extracts generally showed hepatoprotective activities with the reduction of liver enzymes such as ALT, AST, and ALP. In this regard they also proved to be better than the standard, silymarin. The OB-treated groups also had lower urea which signifies improved renal. The results also showed that OB-treated extracts showed reduction in total protein values with significant change in albumin only in the chloroform extract). This suggests a reduction in globulin levels indicating an anti-inflammatory activity. Averagely, the methanolic extract of OB showed the greatest hepatoprotective activity as seen in the lowest ALT value, second lowest AST value, and second lowest urea values. Similarly, the methanolic extract showed the least deviation from the negative control for the serum total bilirubin and D bilirubin values.

Extensive studies have been carried out investigating the hepatoprotective, renoprotective, and neuroprotective activities of the aqueous leaf and seed extracts of other important species of *Ocimum*. The results showed that the leaf extract of *O. sanctum* is hepato-protective against paracetamol as revealed by the significant reduction in the serum enzymes such as AST, ALT, and ALP in rats.

The histopathological examination of the rats' liver revealed the inherent danger of overdose of acetaminophen as this caused severe hepatic damage. *Ocimum basilicum*, at very high dose (3000mg/kg) caused acute hepatic toxicity in the rats as revealed in lesions such as severe diffuse individualization of hepatocytes, hyper-eosinophilic cytoplasm, and nuclei karyorrhexis of hepatocytes. The administration of Silymarin at 100mg/kg did not cause sufficient amelioration as observed in the hepatic lesions as compared with other solvent extracts. This may be attributable to the low dose of silymarin as compared with other solvent extracts. The various solvent extracts at different concentrations of *Ocimum basilicum* administered to the rats reduced the effect of acetaminophen toxicity as the lesions were not as severe as it was without the extract. This agrees the work of Renzulli et al. [39] which proved that the protective effect of *Ocimum basilicum* extract was due to reduced

DNA fragmentation and by the inhibition of caspase-3 activation. Although at 1200mg *Ocimum basilicum* ether extract showed a more severe hepatic injury, the administration of 1000mg/kg of *Ocimum basilicum* ethylacetate extract, and 1000mg/kg of *Ocimum basilicum* methanol extract were more hepatoprotective. This is evidenced in the moderate lesion seen in the liver of these groups of rats. The rats in the group administered with 1200mg/kg of *Ocimum basilicum* chloroform extract showed evidence of hepatic regeneration.

In a similar study, Renzulli et al. [39] demonstrated that Rosmarinic acid is a natural phenolic compound which is present in many herbs of the family *Labiatae* (*Lamiaceae*). This compound is also present in *O. basilicum*, and it is known to inhibit complement-dependent inflammatory processes. In combating oxidative stress, Rosmarinic acid also reduces reactive oxygen species production which inhibits DNA and protein synthesis

CONCLUSION

This study indicates that the chloroform, diethylether, ethylacetate and methanol extracts of *O. basilicum* whole plant exert hepatoprotective effect against acetaminophen-induced hepatotoxicity. The study also shows that the methanolic extract of

this plant has a better hepatoprotective activity compared to the chloroform, diethylether, and ethylacetate extracts as indicated by the lowest values of serum liver enzyme parameters. Thus, the findings support the use of this plant for the treatment of hepatic disorders like jaundice.

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Table 1. Phytochemical Constituents of *Ocimum basilicum*

Phytochemical	Chloroform	Ether	Etyhlacetate	Methanol
Saponin	-	-	+	-
Tanin	+	+	+	+
Steroid	-	+	+	+
Cardiac Glycoside	+	+	+	+
Anthraquinone	-	-	-	-
Flavonoids	+	+	+	+
Alkaloids	-	-	-	-
Terpenes	-	+	+	+

Key: + (present), - (absent)

Table 2. Serum biochemical analytes of rats administered silymarin and solvent extract of *Ocimum basilicum*

Parameter	ALT (U/l)	AST (U/l)	ALP (U/l)	Urea (mmol/l)	Creatinine (μ mol/l)	TBIL (μ mol/l)	DBIL (μ mol/l)	Total Protein (g/l)	Albumin (g/l)
Control groups									
Positive control (acetaminophen)	34.37 \pm 0.13 ^a	50.21 \pm 11.99	0.06 \pm 0.02 ^b	5.02 \pm 0.71	55.28 \pm 5.45 ^{a,b}	1.43 \pm 0.26 ^a	0.82 \pm 0.17	91.86 \pm 6.52 ^b	34.73 \pm 0.78
Negative control (water only)	8.57 \pm 2.06	29.35 \pm 2.96	0.070 \pm 0.013	5.36 \pm 0.77	36.85 \pm 3.93	0.45 \pm 0.08	0.399 \pm 0.09	91.72 \pm 3.00	35.19 \pm 0.05
Standard Control (Silymarin treated)	35.47 \pm 3.07 ^a	50.26 \pm 6.80 ^a	0.145 \pm 0.022 ^a	5.82 \pm 0.17	38.06 \pm 4.59	1.27 \pm 0.11 ^a	1.07 \pm 0.31 ^a	79.31 \pm 4.40 ^b	34.31 \pm 0.46
OB treated group									
Chloroform extract	23.21 \pm 2.3 ^a	40.87 \pm 4.09	0.062 \pm 0.01 ^b	1.83 \pm 0.18 ^{a,b} c	23.14 \pm 2.31 ^{a,b,c}	2.83 \pm 0.28 ^{a,b} c	1.58 \pm 0.16 ^{a,c}	73.92 \pm 7.39 ^{a,c}	15.17 \pm 1.52 ^{a,b,c}
Ethyl acetate extract	20.36 \pm 8.99 ^b	33.47 \pm 2.64 ^b	0.133 \pm 0.049 ^{a,c}	2.06 \pm 0.29 ^{a,b}	44.36 \pm 4.39	1.33 \pm 0.31 ^a	1.25 \pm 0.03 ^a	83.09 \pm 4.58	33.72 \pm 1.01
Diethyl ether extract	17.86 \pm 2.02 ^{b,c}	37.10 \pm 2.89 ^b	0.064 \pm 0.020 ^b	1.60 \pm 0.24 ^{a,b} c	57.86 \pm 5.45 ^{a,b,c}	1.50 \pm 0.34 ^a	1.97 \pm 0.11 ^{a,b} c	86.87 \pm 4.58	34.99 \pm 0.23
Methanol extract	10.42 \pm 2.44 ^{b,c}	36.08 \pm 11.06 ^b	0.112 \pm 0.001	1.74 \pm 0.51 ^{a,b} c	41.78 \pm 3.23 ^c	0.53 \pm 0.08 ^{b,c}	0.51 \pm 0.02 ^b	82.01 \pm 0.76	34.63 \pm 1.01

Superscripts (a, b, & c) indicate significant difference ($p < 0.05$) in each column.

Key: a-Groups II to VII compared with GP I,
GP VII.

b -Groups III to VII compared with GPII,

c -Groups III to VI compared with

CREAT-Serum creatinine

BUN-Blood urea nitrogen

AST-Aspartate

aminotransferase

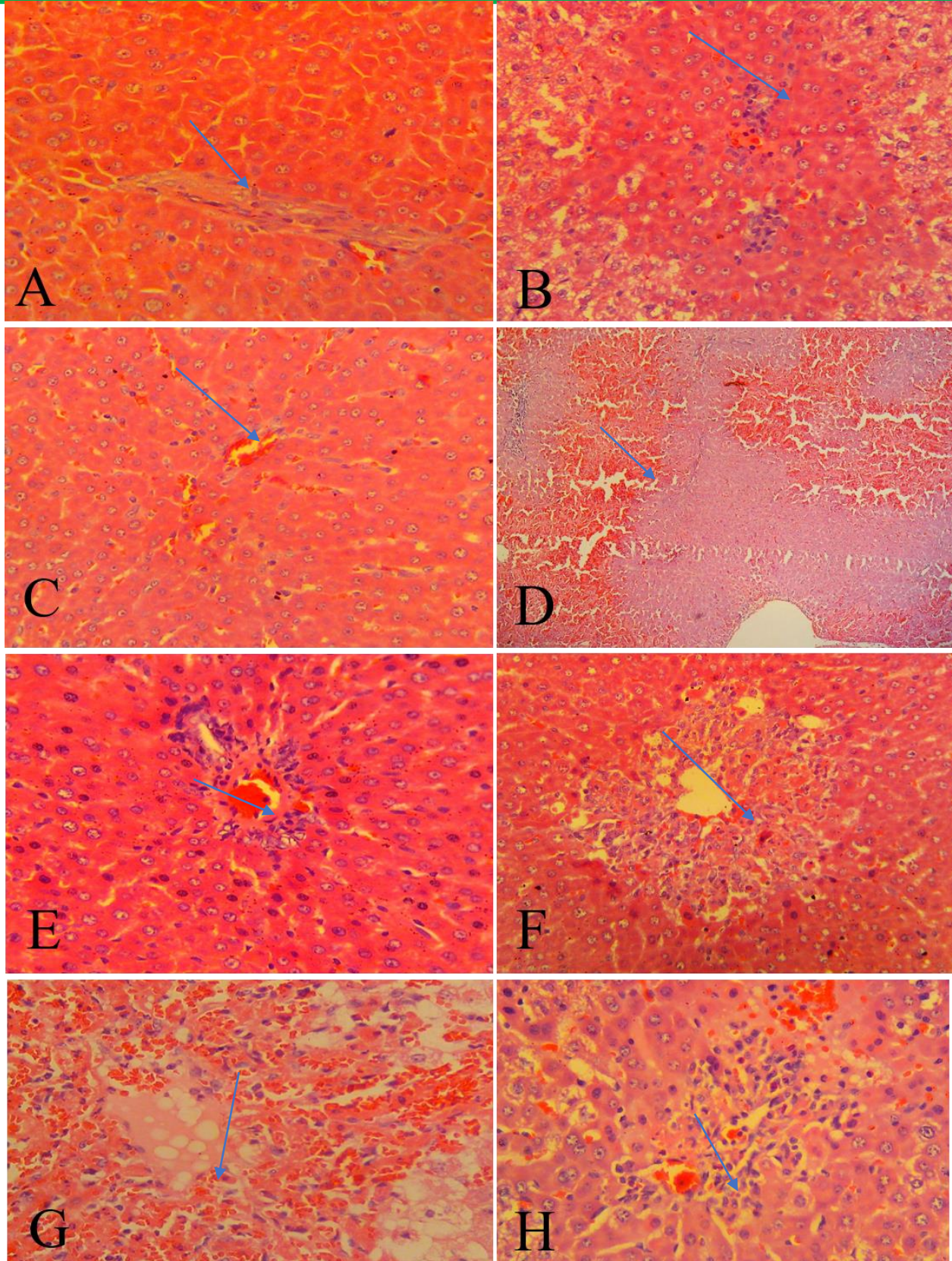
DBIL-Direct bilirubin

ALT-Alanine aminotransferase

ALB-Albumin

ALP-Alkaline phosphatase

TBIL-Total bilirubin



1

- 2 Figure 1: Photomicrograph slides of Liver section treated with Paracetamol and various concentrations of
3 *Ocimum basilicum* fractions
4
5 A: Liver section of rat with severe diffuse hepatocytes individualization, hyper-eosinophilic cytoplasm
6 and nuclei karyorrhexis (hepatocellular necrosis). 3000mg/kg *Ocimum basilicum* acute toxicity x400 HE.
7 B: Liver section of rat with centrilobular and midzonal hepatic necrosis and mononuclear cell infiltration,
8 positive control- 750 mg/kg acetaminophen x400 HE.
9 C: Liver section of rat with centrilobular area, negative control- distilled water x400 HE
10 D: Liver section of rat with severe diffuse centrilobular and midzonal hepatocellular necrosis often times
11 bridging with severe diffuse haemorrhage. 100mg /kg Silymarin (hepato/protective agent) x100 HE.
12 E: Liver section of rat with moderate diffuse hepatocellular nuclei pyknosis and high chromatin density,
13 1200mg/kg *Ocimum basilicum* chloroform extract x400 HE.
14 F: Liver section of rat with moderate diffuse sub-chronic centrilobular and midzonal hepatic necrosis with
15 neutrophils and macrophage infiltration 1000mg/kg *Ocimum basilicum* ethylacetate extract x400 HE.
16 G: Liver section of rat with severe diffuse centrilobular and midzonal hepatic lipidosis (fatty change) and
17 necrosis with severe vascular destruction and haemorrhage, 1200mg *Ocimum basilicum* ether extract
18 x400 HE.
19 H: Liver section of rat with moderate diffuse sub-chronic centrilobular and midzonal hepatic necrosis
20 with neutrophils and macrophage infiltration. 1000mg *Ocimum basilicum* methanol extract x100 HE.
21