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Research Article

Aqueous, Butanolic, Ethanolic and N-hexane fractions of Leaves, Roots, Seeds and Stems of Cajanus cajan and Lycopersicon esculetum downregulated Ki67 and Multidrug resistance1 gene expressions in Ethidium Bromide-induced hepato-toxicity in rats.

AKINLOLU, A.A.[1]*, SULAIMAN, F.A.[2], OLORUNTOBA, O.[3] AND ASOGWA, N.T.[4]
[1]Department of Anatomy, University of Medical Sciences Ondo, Ondo State, Nigeria.
[2]Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria.
[3]Department of Anatomy, University of Ilorin, Ilorin, Kwara State, Nigeria.
[4]Central Research Laboratory, Ilorin, Kwara State, Nigeria.
*Corresponding author:
Email: aadelaja@unimed.edu.ng
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ABSTRACT
Aims: Ethidium Bromide (EB) is an established mutagen/carcinogen. Increased levels of Ki67 and multidrug resistance1 gene (MDR1) are associated with abnormal cellular proliferation and drug resistance respectively. This study examined the effects of aqueous, butanolic, ethanolic and n-hexane fractions of leaves, roots, seeds and stems of Cajanus cajan (CC) and Lycopersicon esculetum (LE) on levels of Ki67 and MDR1 in EB-induced hepato-toxicity in rats.
Methods: 115 adult male Wistar rats were randomly divided into 23 groups (n = 5). 0.5mls of EB solution (0.5g/100mls of ethanol) was applied to scraped ventral skin area of rats. Groups 1 and 2 were treated with Normal Saline and 40mg of Tamsulosin Hydrochloride respectively. Groups 3-11 were treated with 40mg/kg bodyweight of fractions of CC. Groups 12-23 were treated with 40mg/kg bodyweight of fractions of LE. Drugs/extracts were orally administered for 4 weeks. Liver histo-pathology (Heamatoxyline and Eosin technique) and ELISA analyses of Ki67 and MDR1 concentrations were evaluated. Computed data were statistically analysed. Study/analysis was conducted in 2019/2020.
Results: Histo-pathological evaluations revealed normal liver histo-architectures in all Groups. Results showed statistically non-significant lower mean levels (P>0.05) of Ki67 in Groups 3, 5, 6, 8, 10, 13, 14 and 21, compared with Group 1. Analyses showed statistically non-significant lower mean levels (P>0.05) of MDR1 in Groups 5, 6, 9, 10 and 21, compared with Group 1. Therefore, post-treatments with extracts of CC and LE ameliorated EB-induced increased proliferation and drug resistance in rats.
Conclusion: CC and LE possess anti-proliferation, anti-drug resistance and anticancer potentials.
Keywords: Ethidium Bromide, Hepato-toxicity, Ki67, Multidrug resistance1 gene, Cajanus cajan and Lycopersicon esculetum.

INTRODUCTION

Cajanus cajan (CC) (pigeon pea) contains potassium, calcium, vitamins (such as vitamin A), niacin, thiamin, riboflavin, folate and pantothenic acid [1,2]. Lycopersicon esculetum (LE) (tomatoes) contains carotenoids, ascorbic acid, phenolic compounds, α-tocopherol and lycopenes [3]. Lycopene induces phase II enzymes that help to eliminate carcinogens and toxins, thereby protecting lipids, proteins and DNA against cellular toxicity [4]. Lycopene equally inhibits cancer cells proliferation [5], and blocks cell transformation by reducing the loss of cancer cells inhibition contact [6,7]. CC and LE are, therefore, plants of potential therapeutic values and are relevant in the search for anticancer drug compounds in cancer models or studies.

In addition, Ethidium Bromide (3,8-diamino-5-ethyl-6-phenyl-phenanthridinium) (EB) is used to visualize DNA in molecular biology techniques [8]. EB-contaminated compounds or waste materials may leak into water supplies, and consequently contaminate rivers or drinking waters, garden or agricultural products, and possible association with increased incidence of some human cancers have been suspected. EB's toxicity is dependent on the exposed organism and the circumstances of exposure; and it can be absorbed via the skin [8]. EB is an effective intercalator, a strong mutagen [9] and a possible

carcinogen [10]. EB intercalates the double stranded DNA, thereby deforming the molecule and resulting in increased generation of reactive oxygen species, mitochondrial dysfunction, increased cellular proliferation and mutagenesis [11].

Furthermore, cancers comprise of cancer stem cells (CSCs), macrophages and vascular endothelial cells, with CSCs having tumourigenic capacity while others do not [12,13]. Cancer treatment regimens kill most cancer cells, but do not eliminate CSCs, which have protective and resistance mechanisms [12,13] via up-regulation of biomarkers of proliferation (Ki67) and drug resistance (Aldehyde dehydrogenase 1 and P-glycoprotein) [14], upregulation of biomarker of angiogenesis (VEGFR1) [12,13]; down-regulation of apoptotic and tumour suppressor genes (Caspase-3 and p53), as well as de-regulation of proto-oncogenes (myc and src). CSCs are, therefore, able to regenerate other cancer cells well after completion of treatment regimens. Hence, the characteristic survival of CSCs provides explanations for failures of cancer treatments, as well as informed directions for the development of more potent anticancer drugs from plants or other sources.

The multidrug resistance1 (MDR1) gene or P-glycoprotein is localized in the cell-membrane and it functions pharmacologically as an active drug efflux transporter protein of various substances including drugs and toxins [14-16]. The MDR1 protein is physiologically expressed at the bile canalicular membrane of the liver functioning in biliary excretion of lipophilic drugs [17]. The MDR1 protein has affinity for hydrophobic compounds and efforts have been made to by-pass its efflux effect using reversal agents such as R-verapamil, Tween-80 and Cremophor EL. These reversal agents have, however, been reported to induce significant toxicity at required doses for P-glycoprotein's inhibition [14-16]. Ki-67 protein is detected during all the active phases of the cell cycle and it is usually used as a complement to grading systems that include mitotic counting as a sign of proliferation [18-19]. It is one of the five genes (out of 16 cancer-associated genes) of proliferation that is of important weight to the Oncotype score. Ki-67 is not expressed by quiescent or resting cells in the G0-phase, hence it is an excellent operational marker for evaluation of the proliferation of a given cell population and the aggressiveness of malignancies [18-20].

The characteristic abnormal cellular proliferation with accompanied increased expressions of Ki67 and MDR1 by Cancer Stem Cells (CSCs) makes the treatment of cancers a very challenging task. It is, therefore, very relevant to evaluate plants sources towards the isolation of drugs compounds that can specifically target CSCs and reduce or eliminate drug resistance. The liver plays significant roles in detoxification, drug metabolism and the functionality of the body systems. We, therefore, evaluated the effects of the aqueous, butanolic, ethanolic and n-hexane fractions of leaves, roots, seeds and stems of Cajanus cajan and Lycopersicon esculetum on levels of Ki67 and MDR1 gene or P-glycoprotein in the liver tissues of rats in Ethidium Bromide-induced hepato-toxicity in-order to further determine which plant parts possess hepato-protective, anti-proliferation, anti-drug resistance and anticancer potentials.

MATERIALS AND METHODS

Collection of Plant Materials

Freshly cut seeds, stems and leaves of C. cajan; and freshly cut roots, stems and leaves of L. esculetum were obtained from the school forest of University of Ilorin, Nigeria. Attestation and verification of plant materials were conducted at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Nigeria.

Preparation of Plant Extracts

The collected fresh seeds, stems and leaves of C. cajan; and freshly cut roots, stems and leaves of L. esculetum were washed for removal of sand and debris, and then dried under shade at 25-30°C for three weeks. Thereafter, the plant materials were pulverized and homogenized with an electric blender to increase the surface area for solvent extraction before usage in the experimental procedures.

The weight of powdered sample of about 200g of C. cajan and L. esculetum were measured on an analytical balance (Sartorious BS 124S, China) with 0.1mg accuracy. Aqueous extracts were obtained at 50mg/L with distilled water by infusions for 5-10 minutes. The extracts were cooled at 25-30°C and centrifuged at 2000 revolutions per minute (rpm) for 10 minutes in a centrifuge (Centribio, MLW T24 D, Germany) in-order to obtain the supernatant. Thereafter, aqueous extracts were collected following filtration of obtained supernatant with Whatman N0. 4 filter paper.

The butanolic extracts were obtained using modifications of previous procedures as described by Tan et al., 2014 [21]. Solutions from the pulverized dried seeds were prepared at a concentration of 0.1g/ml with 70% ethanol, while

maceration was carried out in the dark for 7 days at 25 ± 2°C. The resulting mixtures were totally dried using a rotary evaporator (Heidolph 4011, Canada). Infusions of the resulting solid were carried out using distilled water to obtain a concentration of 50μg/ml. The resulting extracts were centrifuged at 2000 rpm for 10 minutes, and the supernatants were filtered with Whatman N0 4 filter paper. N-hexane was added to soak the left over for another 72 hours; and separate funnel was used to separate the N- hexane fraction and served to remove the particles.

Equipment and Reagents

Animal cage, weighing balance, sensitive balance, pellet grower mash, water, dissecting set, distilled water, Tamsulosin Hydrochloride, pong capsule microscopic glass slides, cover slip, alcohol, water bath, 10% formosaline, needles and syringes, gloves, face masks, xylene, paraffin wax, DPX mountant, gauze and cotton wool, staining jars, tissue cassette and moulds, ice pads and freezer, water bath, rotary evaporator, microtome and microscope.

Experimental Animals

One hundred and fifteen (115) healthy albino Wistar rats (7 weeks old and with an average weight of 150g) were purchased from the Animal House of the Department of Biochemistry of the University of Ilorin, Nigeria. The animals were kept in standard and ventilated cages, fed with standard rat feed and access to tap water was granted ad libitum in a hygienic environment.

Induction of Ethidium Bromide (EB) Hepato-toxicity

EB (0.5g) was dissolved in 100mls of ethanol. With the aid of a dissecting blade, 7cm width of the skin of each rat was scraped ventrally in the midline from the neck to the pelvic region, and 0.5ml of EB solution was applied to the scraped skin area topically.

Administration of Drugs and Extracts

All 115 rats used in the present study were exposed to 0.5ml of EB solution (0.5g of EB dissolved in 100mls of ethanol). The rats, thereafter, were divided into 23 Experimental Groups as presented in Tables 1 and 2, comprising of 5 rats per group. The number of rats per group was determined in compliance with the policy statements of the University of Ilorin Ethical Review Committee regulating laboratory animal use. For Tables 1 and 2, following EB-exposure on Day 1 of experimental procedure, rats of Control Group 1 and Experimental Group 2 were post-treated orally with Normal Saline and Tamsulosin Hydrochloride (used in the treatment of hyperplasia) respectively for 4 weeks (Days 1 – 28). In addition, following EB-exposure on Day 1 of experimental procedure, 9 Groups (CC3-11) were post-treated orally with 40mg/kg body weight of Aqueous, Butanolic or Ethanolic extracts of the seeds, stems and leaves of C. cajan for 4 weeks (Days 1 – 28) as presented in Table 1. Twelve (12) separate Groups (LE3-14) were post-treated orally with 40 mg/kg body weight of Aqueous, Butanolic, Ethanolic or N-hexane extracts of the roots, stems and leaves of L. esculetum for 4 weeks (Days 1 – 28) as presented in Table 2.

Histo-pathological evaluations of the Liver

Following the completion of the 4-week experimental procedure, each rat was sacrificed using anaesthetization via diethyl-ether inhalation, and the abdominal cavity was exposed. Thereafter, liver tissues were processed for light microscopy using conventional histological procedures. Histo-pathological evaluations of the liver were conducted using Heamatoxyline and Eosin technique as earlier described by Akinlolu et al., 2017 [22].

Enzyme Linked Immunosorbent Assay (ELISA) of MDR1/P-glycoprotein concentrations in Liver tissues of rats

Liver tissues were excised, isolated and homogenized with the aid of porcelain mortar and pestle in 0.25M ice-cold sucrose, with 1g of tissue homogenate prepared in 4ml of 0.25M sucrose solution. The obtained homogenates of liver tissues were topped up to 5ml with 0.25M ice-cold sucrose, and transferred into a 5ml serum bottle. Homogenates were later centrifuged for 15 minutes using a centrifuge (Model 90-1) at 3000 revolution per minute. The resultant supernatant was stored at -4^{O}C, and evaluated for the liver tissues' amount of MDR1/P-glycoprotein in all groups of rats by employing ELISA method.

This ELISA assay uses the quantitative sandwich enzyme immunoassay method. MDR1/P-glycoprotein specific antibody was pre-coated onto a microplate. Samples and Standards were tuneled into the wells while presenting MDR1/P-glycoprotein was bound by the immobilized antibody. A biotin-conjugated antibody specific for MDR1/P-glycoprotein was added to the wells following removal of unbound substances. Thereafter, Avidin conjugated Horseradish Peroxidase was transferred into the wells after

washing. A substrate solution was introduced into the wells resulting in colour development in relationship with the quantity of P-glycoprotein bound in the initial step following removal of any unbound avidin-enzyme reagent via washing. Finally, colour intensity was measured and the development of colour was stopped.

Statistical Analyses

The statistical data acquired from the micro plate ELISA results were analyzed and comparisons between each Experimental Group and Control Group were conducted for any significant difference by employing one-way analysis of variance (ANOVA), while Tukey post-hoc test was employed for groups' comparisons. Statistical level of significance was set at $P \leq 0.05$.

RESULTS

Histo-pathological Evaluations of the Liver in rats of Groups 1 – 23.

Histo-pathological evaluations revealed normal liver histo-architecture in rats of Groups 1 – 23 (Figures 1 – 23). Normal histo-architectures of the liver components were observed such as polygonal epithelia hepatocytes branching to form irregular plates that are separated by venous sinusoids and radiating from the plates of the hepatocytes are the central veins.

Evaluations of Ki67 concentrations in Liver homogenates of rats of Groups 1 – 23.

Results showed statistically non-significant lower mean values ($P>0.05$) of Ki67 in rats of Groups 3 ($P = 0.25$), 5 ($P = 0.40$), 6 ($P= 0.24$), 8 ($P = 0.13$), 10 ($P = 0.57$), 13 ($P = 0.86$), 14 ($P = 0.65$), 21 ($P = 0.63$), when compared with Groups 1 (6.42 ± 0.24) (Table 1). However, there were statistically non-significant higher mean values ($P>0.05$) of Ki67 in rats of Groups 4 ($P = 0.13$), 7 ($P = 0.54$), 9 ($P = 0.08$), 11 ($P = 0.11$), 12 ($P = 0.23$), 15 ($P = 0.13$), 16 ($P = 0.51$), 17 ($P = 0.05$), 18 ($P = 0.01$), 19 ($P = 0.15$), 20 ($P = 0.33$), 22 ($P = 0.12$) and 23 ($P = 0.09$), when compared with Group 1 (6.42 ± 0.24) (Table 1).

Evaluations of MDR1 gene/P-glycoprotein concentrations in Liver homogenates of rats of Groups 1 – 23.

Results showed statistically non-significant lower mean levels ($P>0.05$) of MDR1 in rats of Groups 5 ($P = 0.32$), 6 ($P = 0.45$), 9 ($P = 0.38$), 10 ($P = 0.82$) and 21 ($P = 0.95$), when compared with Group 1 (183.20 ± 36.77) (Table 2). However, there were statistically non-significant higher mean values ($P>0.05$) of MDR1 in rats of Groups 3 ($P = 0.61$), 4 ($P = 0.15$), 7 ($P = 0.32$), 8 ($P = 0.32$), 12 ($P = 0.56$), 13 ($P = 0.23$), 14 ($P = 0.15$), 15 ($P = 0.03$), 16 ($P = 0.25$), 17 ($P = 0.11$), 18 ($P = 0.18$), 19 ($P = 0.24$), 20 ($P = 0.30$), 22 ($P = 0.14$) and 23 ($P = 0.41$, when compared with Group 1 (183.20 ± 36.77) (Table 2).

DISCUSSION

Histo-pathological evaluations revealed normal liver histo-architectures in rats of Groups 1 – 23 (Figures 1 – 23). This implied that the topical administrations of 0.5ml of EB solution (0.5g of EB dissolved in 100mls of ethanol) and further treatments with physiological saline, Tamsulosin Hydrochloride, or extracts of the seeds, stems, roots and leaves of Cajanus cajam and Lycopersicon esculetum did not result in evident histo-pathology of the liver after 4 weeks.

Ki-67 protein is one of the five genes (out of 16 cancer-associated genes) of proliferation that is of important weight to the Oncotype score [18-19]. Ki-67 is not expressed by quiescent or resting cells in the G0-phase, hence it is an excellent operational marker for evaluation of the proliferation of a given cell population and the aggressiveness of malignancies [18-20]. Statistical analyses showed non-significant lower mean levels ($P>0.05$) of Ki67 in Groups 3, 5, 6, 8, 10, 13-14 and 21, compared with Group 1 (Table 1). These results implied that topical administration of 0.5ml of EB solution (0.5g of EB dissolved in 100mls of ethanol) resulted in hepato-toxicity, mutagenesis and increased cellular proliferation as evident by the significant upregulation of Ki67 in rats of Control Group 1 (Table 1). This observation is in agreement with the views of [20], which opined that all proliferating cells tested expressed Ki67, and that there is no evidence to the contrary that proliferating cells do not express Ki67.

However, post-treatments with the ethanolic extracts of seed and leaf, aqueous extracts of leaf and seed and butanolic extract of leaf of Cajanus cajan ameliorated EB-induced hepato-toxicity, mutagenesis and increased cellular proliferation via positive immunomodulation and downregulation of Ki67 in rats. Similarly, post-treatments with the ethanolic extracts of stem and leaf and N-hexane extract of root of Lycopersicon esculetum ameliorated EB-induced hepato-toxicity, mutagenesis and increased cellular proliferation via positive immunomodulation and downregulation of Ki67 in rats. Furthermore, Ki67 is a biomarker of Cancer Stem Cells (CSCs), hence our findings indicate that the seed and leaf of Cajanus cajan possibly possess anti-cancer

compounds that can specifically target and eliminate CSCs. Similarly, our findings indicate that the stem, leaf and root of Lycopersicon esculetum possibly possess anti-cancer compounds that can specifically target and eliminate CSCs.

MDR1 or P-glycoprotein is a cell membrane protein, which by its pharmacological function as an active drug efflux transporter protein enhances drug resistance capacity of Cancer Stem Cells (CSCs) [15-16]. Hence, significant upregulation of MDR1 is characteristic of drug resistant tumours and has been associated with cancer cells survival [14-17]. Results showed statistically non-significant lower mean levels ($P>0.05$) of MDR1 in Groups 5-6, 9, 10 and 21, compared with Group 1 (Table 2). These results implied that administration of 0.5ml of EB solution (0.5g of EB dissolved in 100mls of ethanol) resulted in hepato-toxicity and increased drug resistance via the upregulation of MDRI protein hepato-toxicity in rats of Control Group 1.

However, post-treatments with ethanolic extract of seed, aqueous extract of leaf and butanolic extracts of seed and leaf of Cajanus cajan ameliorated EB-induced hepato-toxicity, increased drug resistance and mutagenesis via positive immunomodulation and downregulation of MDR1 protein in rats. Similarly, post-treatment with N-hexane extract of root of Lycopersicon esculetum ameliorated EB-induced hepato-toxicity, increased drug resistance and mutagenesis via positive immunomodulation and downregulation of MDR1 protein in rats. In addition, MDR1 protein is a biomarker of Cancer Stem Cells (CSCs), hence our findings indicate that the seed and leaf of Cajanus cajan possibly possess anti-cancer compounds that can specifically target and eliminate CSCs. Similarly, our findings indicate that the root of Lycopersicon esculetum possibly possess anti-cancer compounds that can specifically target and eliminate CSCs.

CONCLUSION

The findings of this study indicate that the seed and leaf of Cajanus cajan possess anti-proliferation, anti-drug resistance and anticancer potentials. Similarly, our findings indicate that the root, stem and leaf of Lycopersicon esculetum possess anti-proliferation, anti-drug resistance and anticancer potentials. It is, therefore, recommended that further studies be carried out to isolate the active ingredients responsible for the anti-proliferation, anti-drug resistance and anticancer potentials of Cajanus cajan and Lycopersicon esculetum.

Ethical Approval

Ethical approval for this study was sought and received from the University of Ilorin Ethical Review Committee with ethical approval number - UERC/ASN/2019/1820. This research study was conducted in accordance with the guidelines for laboratory animal use and care as provided in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and the US guidelines (NIH publication #85-23, revised in 1985).

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Conflict of Interest

The authors have no conflicts of interest to report.

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Informed Consent

Not applicable.

Authors' Contributions

Conception and design: AAA, SFA, OO and ANT.

Critical revision of the article for intellectual content: AAA, SFA, OO and ANT.

Final approval of the article: AAA, SFA, OO and ANT.

Obtaining of funding: AAA, SFA, OO and ANT.

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Collection and assembly of data: AAA, SFA, OO and ANT.

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Table 1: Liver concentrations of Ki67 (Mean±SD) (ng/ml) in rats of Groups 1 – 23.

S/N	Groups	Mean ±SD (ng/ml)	P-value at $P \leq 0.05$ Group 1 versus Groups 2 - 23
1	5g/100mls of EB + 0.5mls of NS	6.42±0.24	
2	5g/100mls of EB + 40mg/kgbw of TH	23.56 ±1.82	0.05*
3	5g/100mls of EB + 40mg/kgbw of CCEL extract	3.75 ±1.55	0.25
4	5g/100mls of EB + 40mg/kgbw of CCES extract	12.81 ±1.90	0.13
5	5g/100mls of EB + 40mg/kgbw of CCES extract	4.85 ±1.58	0.40
6	5g/100mls of EB + 40mg/kgbw of CCAL extract	4.02 ±1.30	0.24

7	5g/100mls of EB + 40mg/kgbw of CCAS extract	8.15 ±1.98	0.54
8	5g/100mls of EB + 40mg/kgbw of CCAS extract	5.57 ±0.07	0.13
9	5g/100mls of EB + 40mg/kgbw of CCBS extract	8.07 ±0.17	0.08
10	5g/100mls of EB + 40mg/kgbw of CCBL extract	5.35 ±1.89	0.57
11	5g/100mls of EB + 40mg/kgbw of CCBS extract	19.21 ±3.16	0.11
12	5g/100mls of EB + 40mg/kgbw of LEER extract	19.45 ±6.86	0.23
13	5g/100mls of EB + 40mg/kgbw of LEEL extract	6.08 ±2.09	0.86
14	5g/100mls of EB + 40mg/kgbw of LEES extract	5.26 ±2.64	0.65
15	5g/100mls of EB + 40mg/kgbw of LEAR extract	19.53 ±3.74	0.13
16	5g/100mls of EB + 40mg/kgbw of LEAL extract	10.26 ±5.66	0.51
17	5g/100mls of EB + 40mg/kgbw of LEAS extract	12.39 ±0.65	0.05
18	5g/100mls of EB + 40mg/kgbw of LEBR extract	42.54 ±0.31	0.01**
19	5g/100mls of EB + 40mg/kgbw of LEBL extract	11.69 ±1.79	0.15
20	5g/100mls of EB + 40mg/kgbw of LEBS extract	7.47 ±0.82	0.33
21	5g/100mls of EB + 40mg/kgbw of LENR extract	5.57 ±1.79	0.63
22	5g/100mls of EB + 40mg/kgbw of LENL extract	31.81 ±6.56	0.12
23	5g/100mls of EB + 40mg/kgbw of LENS extract	20.52 ±2.88	0.09

Table 2: Liver concentrations of MDR1 (Mean±SD) (ng/ml) in rats of Groups 1 – 23.

S/N	Groups	Mean ±SD (ng/ml)	P-value at P ≤ 0.05 Group 1 versus Groups 2 - 23
1	5g/100mls of EB + 0.5mls of NS	183.2±36.77	
2	5g/100mls of EB + 40mg/kgbw of TH	539.2 ±22.63	0.06
3	5g/100mls of EB + 40mg/kgbw of CCEL extract	309.2 ±254.60	0.61
4	5g/100mls of EB + 40mg/kgbw of CCES extract	370.2 ±49.50	0.15
5	5g/100mls of EB + 40mg/kgbw of CCES extract	134.2 ±9.90	0.32
6	5g/100mls of EB + 40mg/kgbw of CCAL extract	123.2 ±62.23	0.45
7	5g/100mls of EB + 40mg/kgbw of CCAS extract	275.2 ±62.23	0.32
8	5g/100mls of EB + 40mg/kgbw of CCAS extract	355.2 ±127.30	0.32
9	5g/100mls of EB + 40mg/kgbw of CCBS extract	142.2 ±15.56	0.38
10	5g/100mls of EB + 40mg/kgbw of CCBL extract	172.2 ±41.01	0.83
11	5g/100mls of EB + 40mg/kgbw of CCBS extract	386.2 ±244.70	0.45
12	5g/100mls of EB + 40mg/kgbw of LEER extract	543.2 ±56.57	0.56
13	5g/100mls of EB + 40mg/kgbw of LEEL extract	256.2±9.90	0.23
14	5g/100mls of EB + 40mg/kgbw of LEES extract	48.2 ±26.87	0.15
15	5g/100mls of EB + 40mg/kgbw of LEAR extract	1261 ±67.88	0.03*
16	5g/100mls of EB + 40mg/kgbw of LEAL extract	389.2 ±113.10	0.25
17	5g/100mls of EB + 40mg/kgbw of LEAS extract	565.2 ±82.02	0.11
18	5g/100mls of EB + 40mg/kgbw of LEBR extract	2962 ±1119	0.18
19	5g/100mls of EB + 40mg/kgbw of LEBL extract	351.2±84.85	0.24
20	5g/100mls of EB + 40mg/kgbw of LEBS extract	246.2±26.87	0.30
21	5g/100mls of EB + 40mg/kgbw of LENR extract	178.2±80.61	0.95
22	5g/100mls of EB + 40mg/kgbw of LENL extract	1966 ±555.8	0.14
23	5g/100mls of EB + 40mg/kgbw of LENS extract	489.2 ±328.1	0.42

Legends for Tables 1 and 2:
EB = Ethidium Bromide, NS = Normal Saline, bw = bodyweight
TH = Tamsulosin Hydrochloride,
CCEL = Cajanus cajan Ethanol leaf extracts,
CCES = Cajanus cajan Ethanol stem extract,
CCES = Cajanus cajan Ethanol seed extract,
CCAL = Cajanus cajan Aqueous leaf extract,
CCAS = Cajanus cajan Aqueous stem extract,
CCAS = Cajanus cajan Aqueous seed extract,

CCBS = Cajanus cajan Butanol seed extract,
CCBL = Cajanus cajan Butanol leaf extract,
CCBS = Cajanus cajan Butanol stem extract,
LEER = Lycopersicon esculetum Ethanol root extract,
LEEL = Lycopersicon esculetum Ethanol leaf extract,
LEES = Lycopersicon esculetum Ethanol stem extract,
LEAR = Lycopersicon esculetum Aqueous root extract,
LEAL = Lycopersicon esculetum Aqueous leaf extract,
LEAS = Lycopersicon esculetum Aqueous stem extract,
LEBR = Lycopersicon esculetum Butanol root extract,
LEBL = Lycopersicon esculetum Butanol leaf extract,
LEBS = Lycopersicon esculetum Butanol stem extract,
LENR = Lycopersicon esculetum N-hexane root extract,
LENL = Lycopersicon esculetum N-hexane leaf extract, and
LENS = Lycopersicon esculetum N-hexane stem extract.

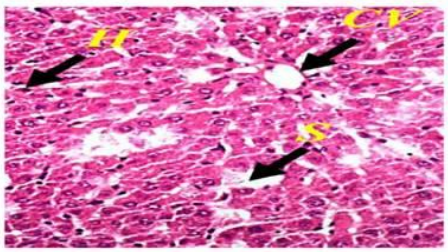

Figure 1.

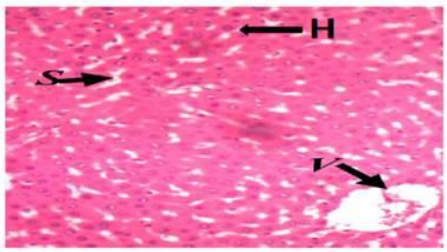

Figure 2.

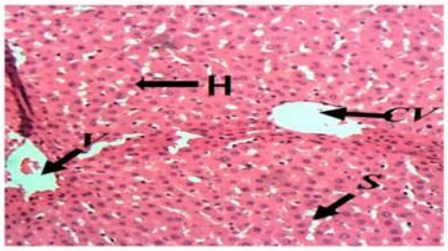

Figure 3.

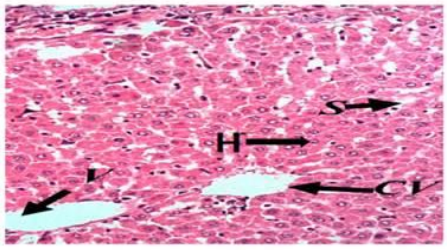

Figure 4.

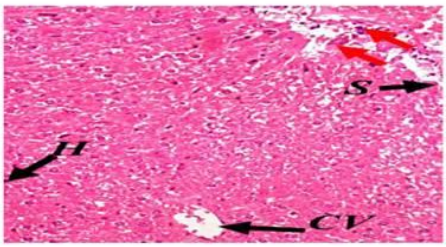

Figure 5.

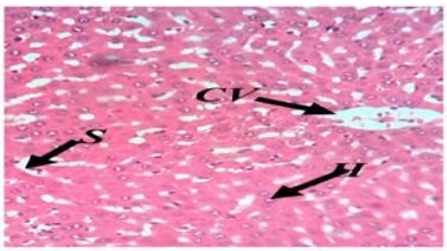

Figure 6.

Fig.1: Photomicrograph sample of the liver of rats of Negative Control Group 1, which received topical administration of 5g/100mls of Ethidium Bromide and were treated with Normal Saline. Magnification: Hematoxylin and Eosin X 100. Scale Bar: 50µm.Normal histo-architectures were observed with polygonal epithelia hepatocytes (H) branching to form irregular plates that are separated by venous sinusoids (S) and radiating from the plates of the hepatocytes are the central veins (CV).

Fig.2: Photomicrograph sample of the liver of rats of Positive Control Group 2, which received 5g/100mls of Ethidium Bromide and were treated with 40mg/kg bodyweight of Tamsulosin Hydrochloride. Magnification: Hematoxylin and Eosin X 100. Scale Bar: 50µm. Normal histological features of the liver components: hepatocytes (H), venous sinusoids (S), central veins (CV), peripheral veins (PV) and veins (V) were observed.

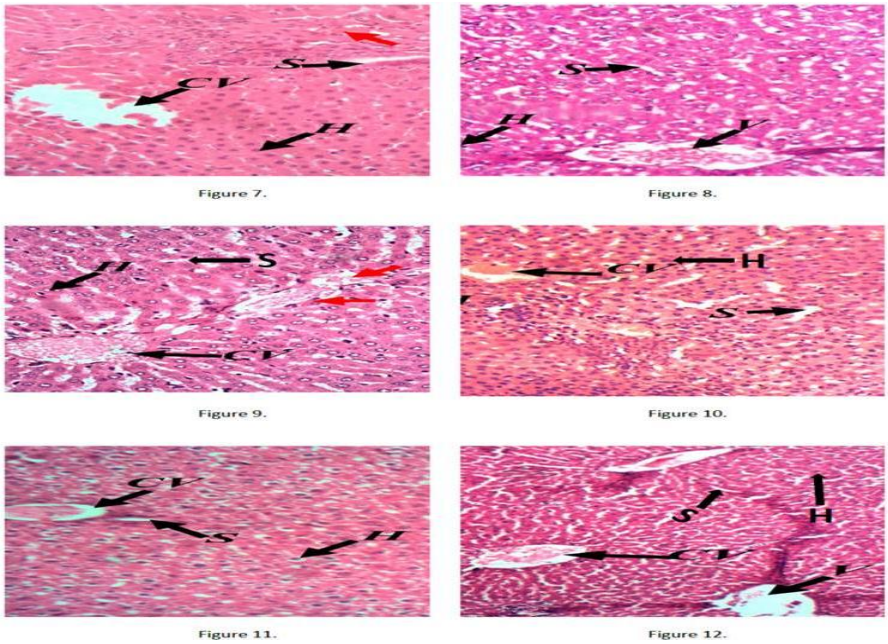

Figure 7. Figure 8. Figure 9. Figure 10. Figure 11. Figure 12.

Fig.3: 11: Photomicrograph sample of the liver of rats of Experimental Groups 3 – 11, which received 5g/100mls of Ethidium Bromide and were treated with 40mg/kg bodyweight of Aqueous, Butanolic or Ethanolic extracts of the Seeds, Stems and Leaves of Cajanus cajan. Magnification: Hematoxylin and Eosin X 100. Scale Bar: 50µm. Normal histological features of the liver components: hepatocytes (H), venous sinusoids (S), central veins (CV), peripheral veins (PV) and veins (V) were observed.

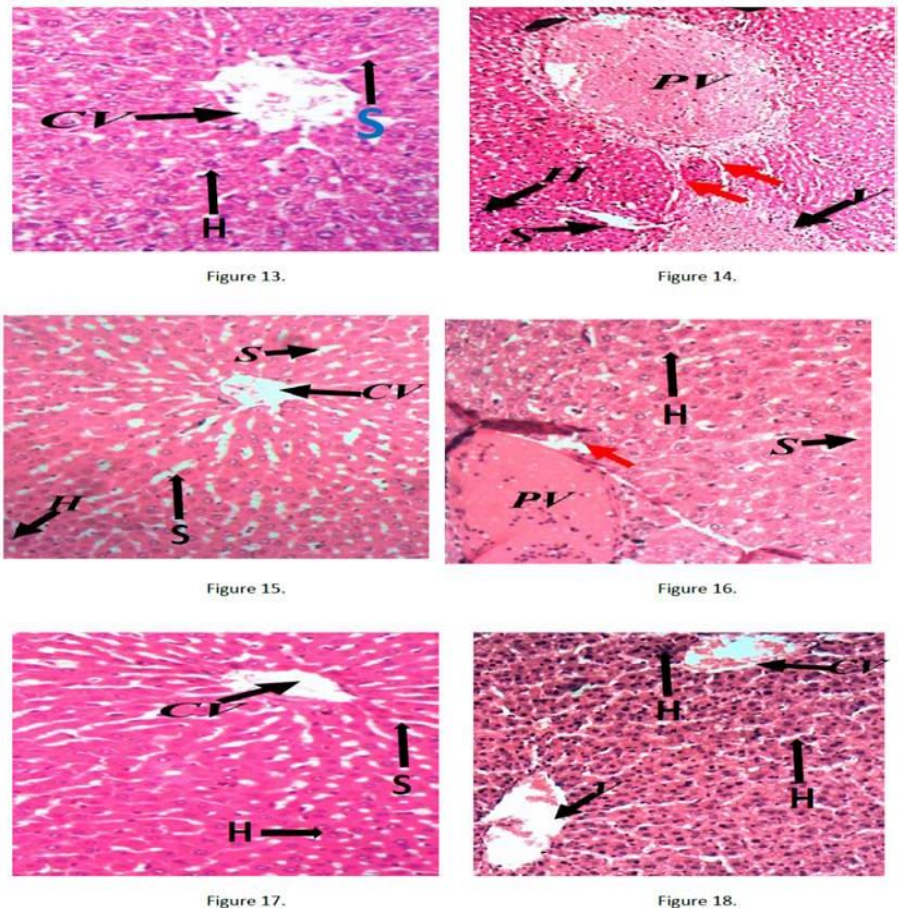

Figure 13. Figure 14. Figure 15. Figure 16. Figure 17. Figure 18.

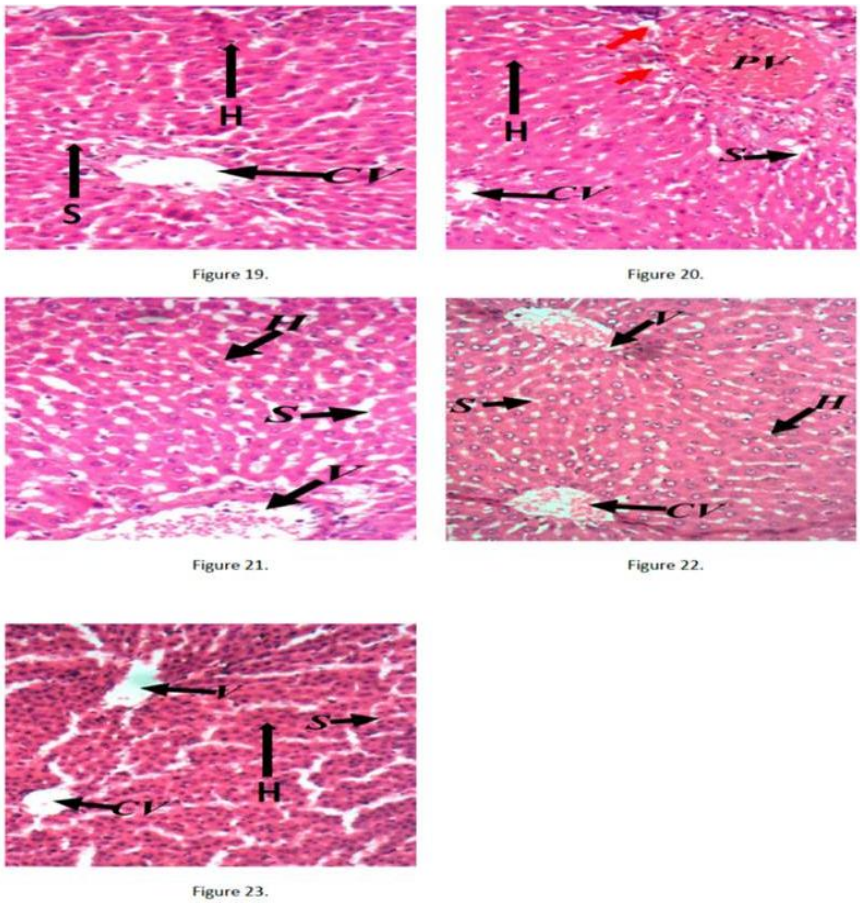

Fig.12 – 23: Photomicrograph sample of the liver of rats of Experimental Groups 3 – 11, which received 5g/100mls of Ethidium Bromide and were treated with 40mg/kg bodyweight of fractions of Aqueous, Butanolic, Ethanolic or N-hexane extracts of the Roots, Stems and Leaves of Lycopersicon esculetum Magnification: Hematoxylin and Eosin X 100. Scale Bar: 50µm.
Normal histological features of the liver components: hepatocytes (H), venous sinusoids (S), central veins (CV), peripheral veins (PV) and veins (V) were observed.