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Reprogramming Cancer Cells using some Biotechnology applications for *Jatropha curcas*.

By: Ahmed N. M. El-atla [1], Warda Y. Ali [1], Mohamed N. Al-Attala [1], Rowyda A. M. Khafaga [2].

Researcher - Environment and Arid Land Agriculture Division, Desert Research Center- Egypt [1].

Dr. Assistant, Faculty of Agriculture, Suez Canal University, Ismailia- Egypt [2].

Email: Ahmed_n982@yahoo.com

Summary

Jatropha plant is known of biofuel production in addition to its medicinal importance in the field of curative drugs for incurable diseases. Jatropha plant has recently been a growing concern in the biofuel sector, in addition to its medicinal importance in curative medicines for incurable diseases. Jatropha seed extract was used in control concentrations 10, 20, 60 and 80 mg / L, respectively, to study its effect on liver Cancer under In vivo conditions. The results concluded that the concentration of 80 mL / L had suppressed and inhibited the cancerous swelling and the ratios of ALT, AST, CEA and Feto-protein = 27.3, 40, .21 and .02, respectively. While at a concentration of 20 mg per liter, no positive change in cancer swelling was observed, but it was observed that at concentrations of 20 and 60 mg / L a change was observed to inhibit the activity of cancer swelling, but it was an effect that did not stop the growth of cancer swelling completely.

Keywords: biofuel, Antioxidant, Jatropha curcas, ALT, AST, CEA.

1. Introduction

In addition to diverse parts to Jatropha have therapeutic value, for example anti-cancer properties (Duke 1983). Roots and Vegetable parts may be used of make antibiotics and also to products to the therapy to skin Natural products diseases (Henning 2002).It is important for impact study to Jatropha extract for the disease to the patients as a Cancer disease for utility maximization and the return on investment to the Jatropha plant, which will benefit of the exploitation to available resources and inputs with no damage for the environment as it is the use to nature with out the use to therapeutic that have hurtful aspects to Human health(Dangambo M.A.*at el* ,2015) . In addition to the above the seriousness to the disease and its spread, this is the reasons major financial burdens of the state and to patients ,and the need has show to reaching for most suitable and also to safe method to therapy(Adam S.E.I *at el* ,1975), which have become very lowering for the third world in special and also to the world of general., where, there was a link between advance and also to well being to societies and the spread of Cancer and that to necessary for find a cure acceptable of industrial the consumer at a reasonable cost.

Cancer is spread and many types depending on where it occurs in the body of the living organism,and the study focused on liver cancer and we followed the method of laboratory experiments on experimental mice because the physical composition of mice closely resembles the physical composition of humans and the proportions used in the experiment are carefully selected from research on the use of Jatropha seed extract in the treatment of cancer in Cell line [Ahmed N.M. *at el* ,(2016)].

2. Materials and methods:

The present study was carried out at the laboratory Animal, Animal Department, Faculty of Veterinary Medicine, Banha University,In addition to Egypt and Medical and Chemistry Laboratory for Doctor Ahmed Matar, Port Said, Egypt. During the period from 2019 to 2020.

2. A- Biochemical analysis:

2. A.1. Plant Materials

The sample (as a replicates) of the seeds from each *Jatropha curcas* were taken to assessment of the physical and chemical properties for Jatropha seeds Extract under study.

2. A.2- Animals :

Twenty five albino Wistar mice (25-35 g) obtained from College of Veterinary Medicine, Banha, Egypt were used for the experiment. The animals were kept under standard laboratory conditions for temperature (25±2°C) and 12/12 h light/dark cycle. They received trade rodent diet (Standard Top® feed) and tap water ad libitum. They were used to the study after one week of Adaptation. Animal Processing was in strict Commitment with Institutional and International guidelines for auspices and use of Animals in Scientific Research [WMA and APS., 2002].

2.A.3. Extraction water from the Jatropha Seeds:

The seeds were dried in the shade and then powdered using a Wiley mill (60-mesh size). To prepare the defatted seed meal, powdered seed material (100 g) was extracted with *n*-hexane (1 L) in a Soxhlet extractor for 6 h at 60°C (AOAC 1960). Then, the defatted seed meal was dried for further extraction by hot water of phytochemicals and biological evaluation.

In order to prepare the hot water extract, 20 g seed powder was mixed with 200 mL hot water (Merck Co. Darmstadt, Germany) and macerated at intervals for 3 days at room temperature 25±2°C. The extract was filtered through Whatman No.1 filter paper and concentrated using a 3.thick, gummy extract obtained was stored in a sealed bottle at 4°C until further use in the experiments. The yield of dry extract (%) was determined in terms of air-dried weight of seed material. The total yield of the hot water extract was 2.5%.

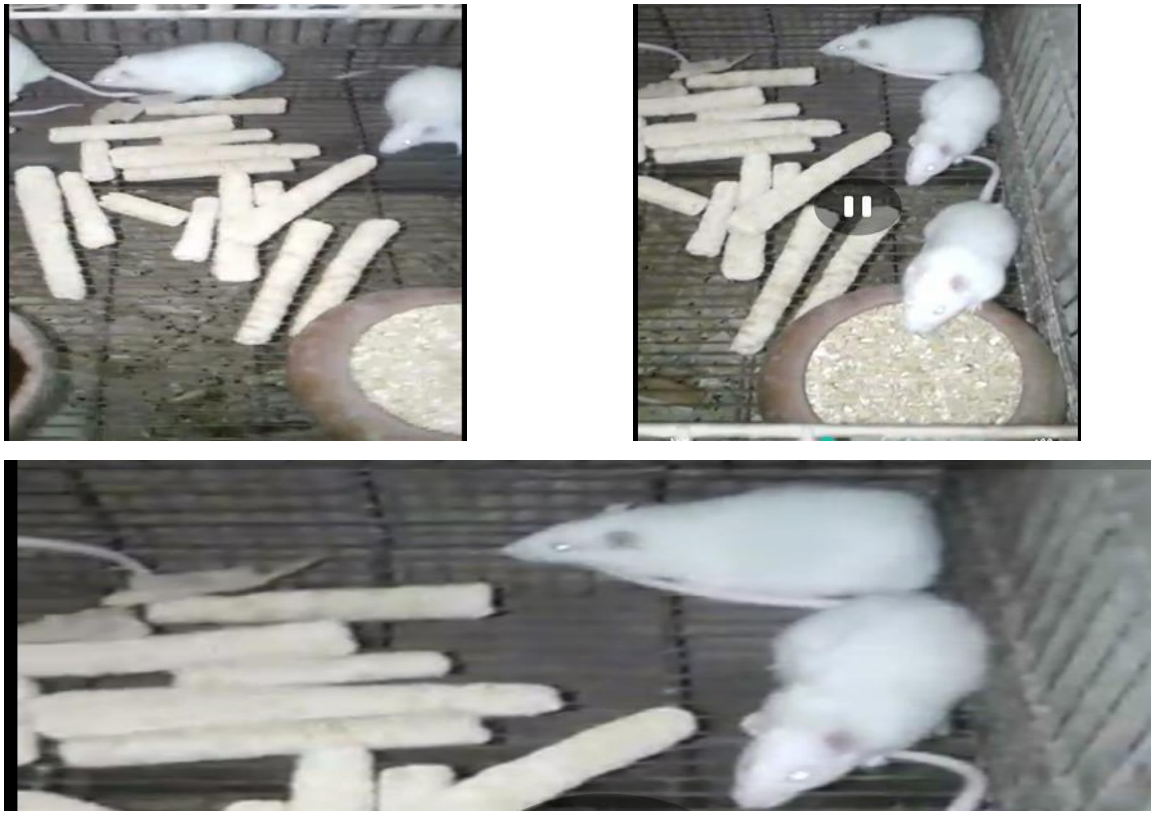
2.A.3.1. Effect of Jatropha seeds extract on cancer cells as Anticancer to Mice:

The current experiment aims to study the effect of different concentration of Jatropha seeds extract on the The liver tissues infected by cancer disease to Mice.

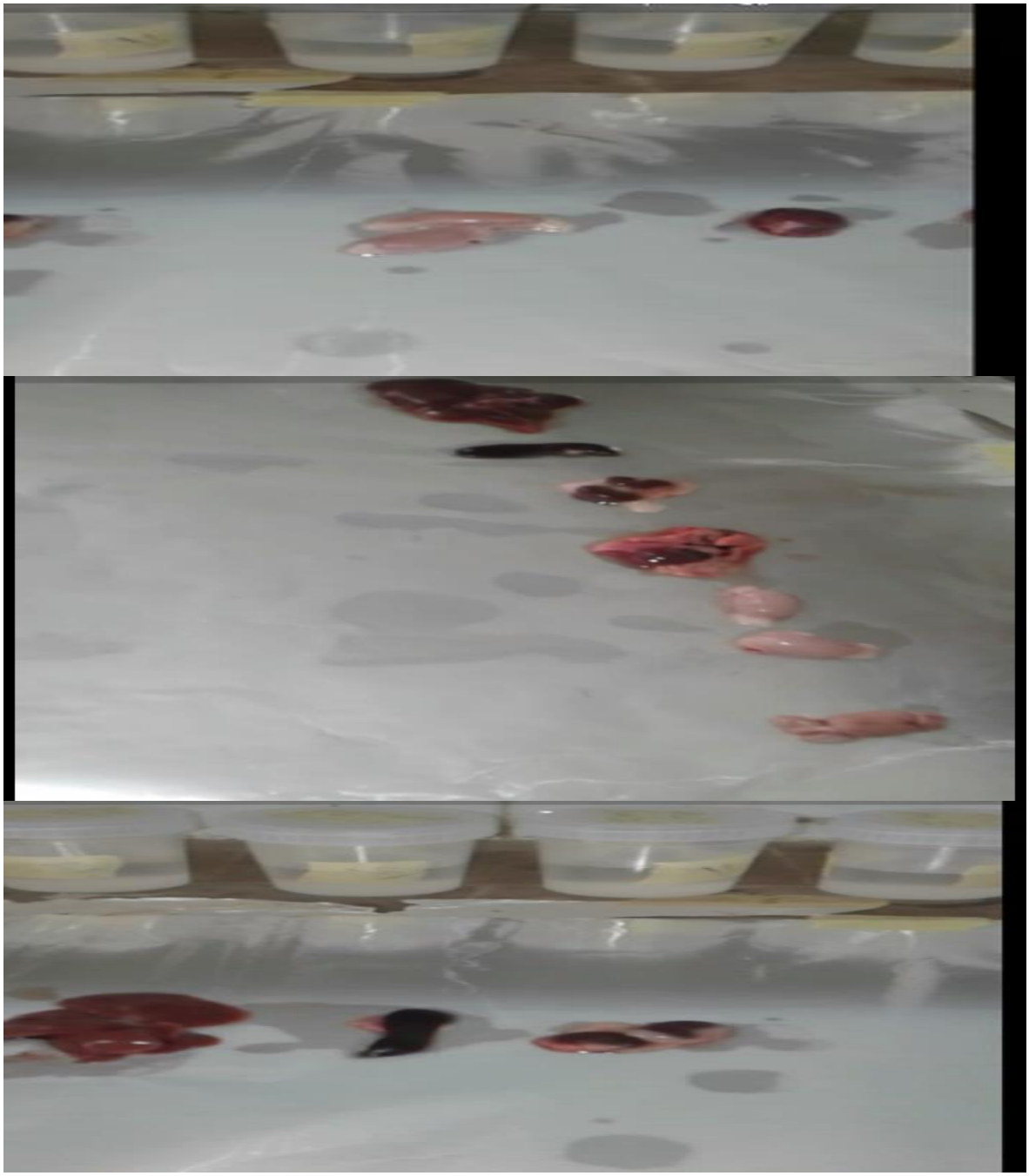
Table (1): Use different concentrations of jatropha extract to inhibit and inhibit cancer.

N	The Concentration mg/L
1	Control
2	10
3	20
4	60
5	80

Fug (2): Stage -1-Mice before cancer.



Fug (3): Stage -2- Mice after cancer**.**



Fug (4): Stage -3- Mice during sampling to measure the effect of the extract on cancer*

* [Heart sections, Liver section, Kidney section, Stomach section, Lungs section and Spleen section in normal control and Jatropha curcas extract treated mice respectively (H and E Stain: x400) congestion of the vessels and interstitium].

2.A.3.2- Chemicals preparation :

Diethyl nitrous amine 2mL acompress that causes liver cancer.

2.A.3.3- Statistical analysis :

All data were tabulated, calculated and statistically analyzed using the computer program SPSS software for windows version 22.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp) Descriptive statistics was calculated in the form of Mean ± Standard deviation (SD).

2.B - samples Clinical For mices and nature of samples:

Multiple organs of rat submitted for histopathological evaluation so sections of fabric strips are equipped rat liver 1.5×1.5×0.5cm, two testicles 1.5 × 0.5 cm each , two kidneys1.5×1cm, each,spleen4×0.5cm,lung 2.5×1.5×0.5cm,brain 2.5×1.5×0.5cm.

3. Results:

3.1. Clinical signs:

The clinical marking spotted were tremors and to vomiting, anorexia, In addition to weakness, diarrhea and too the death. As for the number to deaths spotted during 24 hour is as shown for Table (1).

3.2. Determination of intraperitoneal LD50:

$$LD\ 50 = \sqrt{(a \times b)}$$

Found that to the lowest dose that killed and it caused a defect in the cellular programming to cells dosage given to mice rate to [200 mg kgG1 b.wt.]a, b is highest dose that to cuse kill mouses and it did not adversely affect cell programming [100 mg kgG1 b.wt.], that to the intraperitoneal LD50 to *Jatropha curcas* = 141.4 mg kgG1 b.wt.

3.3. Gross and histopathological findings:

The macroscopical lineaments to maximum to the organs removal for surviving alive mice to both stage one and two did not detect conspicuous

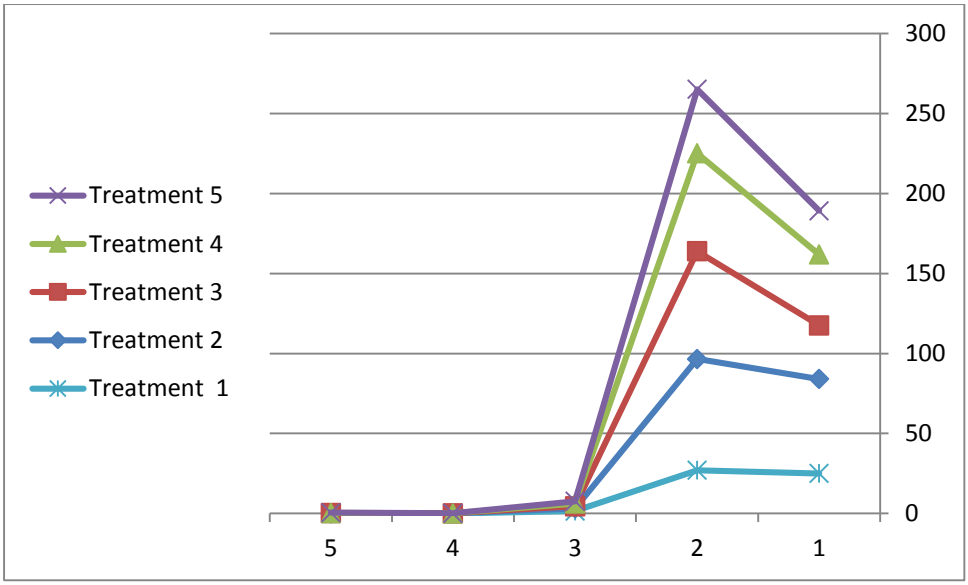
changes.Microscopical screening to the control partition to all Researchs organs explained normal histoarchitecture, also that dose-dependent histomorphological derangements to organs to the cured mice were spotted (Fig. 1a-l). Liver: [ruptured vessels and hemorrhage and also to inflammatory cellular infiltration (Fig. 1)].

Table (2): Percentage mortality within 24 h in mice given Jatropha seed extract at different doses:

	Collection one			**Collection two**			**Collection three**			**Collection fourth**		
Groups	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Dose(mg 1kgG)	**C**	**C**	**C**	**10m/L**	**10m/L**	**10m/L**	**20m/L**	**20m/L**	**20m/L**	**60m/L**	**60m/L**	**60m/L**
No. of animals	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
No. of death	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Mortality (%)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table (3): Effect of Jatropha seed extract on cancerous liver function.

N	GPT/ALT (0-50u/L)	GOT/AST (0-50u/L)	ALK 1u	CEA 3ng/ml or less	Feto-Protein 10n/g or less
1	25	27	1.5	0. 11	0.01
2	59c	69.6c	1.7c	0.11c	0.16c
3	33.6b	67.3b	1.3b	0.09b	0.01b
4	44.3b	61.3b	1.7b	0.09b	0.01b
5	27.3a	40a	1.4a	0.2a	0.12a
Max	59	96.3	1.7	0.2	0.16
Min	25	43.3	1.3	0.09	0.01
Variance	214.6952381	679.6666667	5493.714	0.005111	0
SD	14.65248232	26.07041746	74.11959	0.071494	0
Ave	37.86666667 14.31301587	66.66666667 45.31111111	191 366.2476	0.1475 0.000341	0.06 0
SE	3.783254667	6.731352844	19.1376	0.01846	0



The fug (5): The schematic figure shows that treatment (5) was the most and closest normal ratios of different liver enzymes.

3.4. Clinical picture and nature of specimens:

Sections to samples under examination (1,2,3 and 4) examined from brain, kidneys, testis, liver and spleen show unremarkable histologic feature apart from the liver that shows focal nodular hyperplasia and also, the sections examined from the lung shows congested alveoli and areas of moderate inflammatory infiltrate formed of lymphocytes, plasma cells. few neutrophils and foamy histiocytes. Pathological tests of the samples under test showed that the sample 5(80mg/L) achieved curbing the spread of cancer in the liver tissue and the cancerous cells were eliminated, as shown in Table (3) that anticancer agents and indications of cancer in the sample 5(80mg/L) are at the normal level and while it is clear from the pathological test and tumor markers for the sample 2(10mg/L), as in Table (3), that cancer is still present, but tests for tumor markers indicate slowing down of cancer spreading activity ,and also, it appears from the pathological test and oncological evidence that samples 3(20mg/L) and 4(60mg/L) indicate results in inhibiting and inhibiting the growth of cancer cells to a lesser degree than sample 5(80mg/L),

And were better in controlling cancer than sample 2(10mg/L) and less inhibiting effect of the growth and spread of cancer than sample 5 (80mg/L). Compared to sample (1), the mice did not have cancer, so liver enzymes were at their normal level, and cancer evidence testing was used to confirm cancer-free.

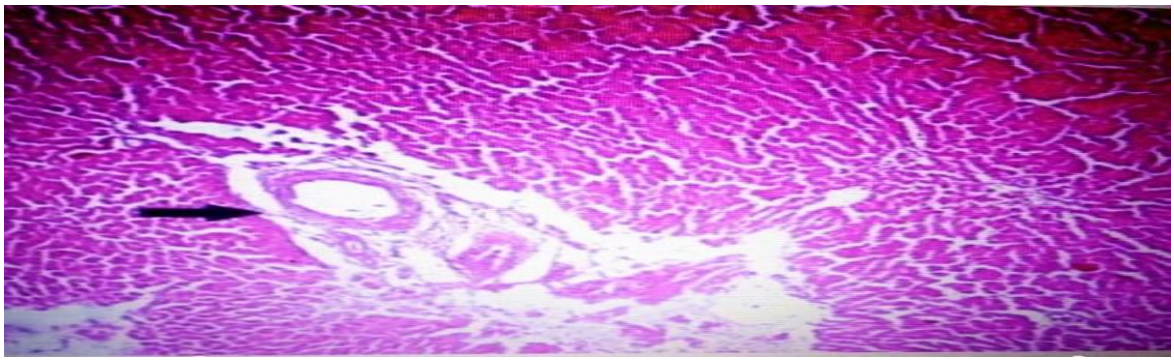


Photo (1):

Aphotomicrography to control (normally) showing normalally hepatic tissue, and also was The liver was divided histologically into lobules*.

*Cell normally to the lobule was the central vein (thin arrow) and also to the periphery to the lobule there are the portal triads (thick arrow) (H&E100).

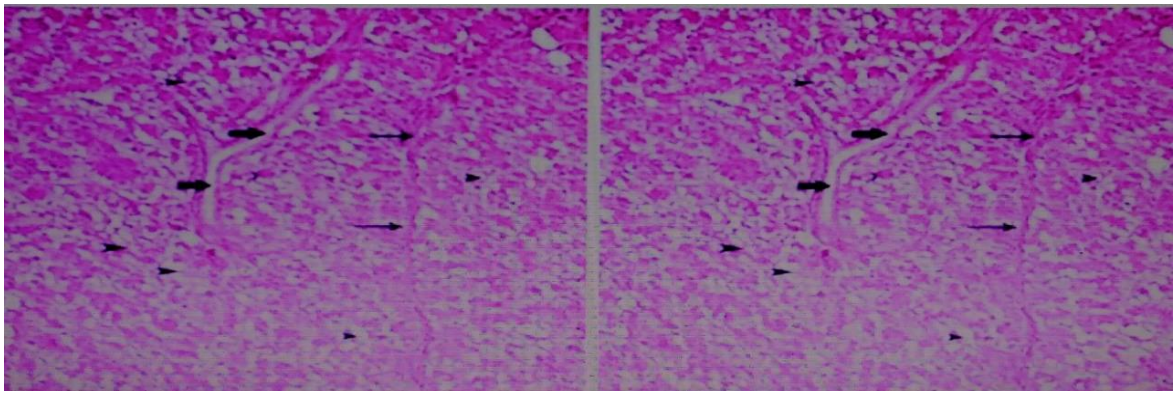


Photo (2):

Aphotomicrography to Cancer affected rats (treatment concentration of 10 mg / L)* *hepatic tissue, the left one showing portal tract (P.T) and radiating fibrous strands (arrows),

while the right one with highly magnification showing the fibrous stands (thin arrow) and ballooning degenerative hepatic cells (arrow heads) all this lead to compressed blood vessels (thick arrow (H&E 100) , so that All of the above is due to the proliferation of cancerous cells in the liver tissue.

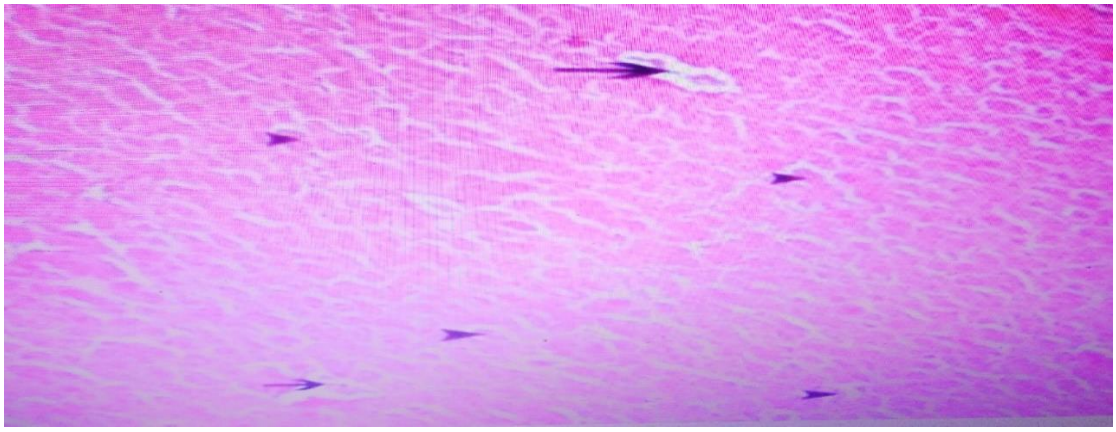


Photo (3):

Tomography of the treatment effect of 80 mg / L jatropha seed extract for laboratory rats showing central veins (arrows) and hepatocytes (arrowheads) improved (H&E 100) and as it appears to suppress the cancerous growth caused by defect in cell programming.

3.6. Discussion:

Search to available literature discover paucity to information in connection with the acute toxicity to *Jatropha curcas* seeds despite the common use to the plant for some herbal therapy. In the results to the present study: the intraperitoneal dosage to the crude extract for *Jatropha curcas* seeds output dosage dependent organ toxicities inclusive the heart, liver, kidney, lungs and spleen. The LD50 It was received being 141.4 mg kgG1 b.wt. Point out that the substance may be believe and treat felled for health according for [Clark and Clark., (1977)]. This is to great significance to view to the wide spread use to *Jatropha curcas* plant and hence must be a matter for concern.

A previous Search certified an oral LD50 to rats for nearly 2793 mg kgG1 and also this was count a little toxic [Dangambo *et al*., (2015)].

It is known that drugs/biological elements when taken orally tolerate events that decrease the amount connect Blood circulation system to pharmacological impacts [Brander *et al*., (1991)]. The clinical signs to toxicity demonstrated to the present study must be linked for a single or Compound effects to the toxic basis present for the extract. Thus, these aggregate the effects indicate a low safety margin to the aqueous extract to *Jatropha curcas* seeds. The credibility to LD50 as the final tool to the limitation to toxicity is prevent as there is a wide difference for results between various species and even tothe same species under various experimental case [Dapar *et al*., (2007)]. Moreover, induction LD50 value acquired to a specie to other species presents enormous wonder and does not backed up information of the particular system washout that led to death [Zbinden and Flury R., (1981)]. In the current study, therefore and the histopathological belongings spotted for the liver organ studied are direct or indirect restraint for toxic standards to *Jatropha curcas* seed extract. Curcin, diterpenes and phenolic elements, phytates and also to trypsin controllers are the toxic rules detected to *Jatropha curcas* [Shukla *et al*., (2015)]. The spotted changes to the histoarchitecture to the organs are comparable those authenticated to a former report [Adam and Magzoub., (1975)] which detected hemorrhage to Nubian goats Animals visceral organs after of poisoning, with *Jatropha curcas* seeds. Also to many Researchs have shown gross and also histological modification to the heart, lungs, kidney, liver and spleen after *Jatropha curcas* administration to a dose following manner [Adam, 1974; Abdel G., *et al*., 2003]. In our studying, the inflammatory cellular permeation spotted to tissues suggests outstanding restraint to the tissues to detrimental impacts [El-Banhawy *et al*., (1993)]. More so, the lesions spotted to the renal tissue describe a good picture to the nephrotoxic quality to the plant. Also that to some aromatic and medicinal plants to nephrotoxic properties of matter have been reported [Dapar *et al*., 2007; Azubike *et al*., 2009], and it is due to the deaths recorded of our work mast be related max to these structural tissue perturbation spotted.

The major toxins to the seeds and Seed oil content to *J. curcas* are phorbol esters. These toxins are also found to be present to the leaves, flowers, roots and also are stems to the plant. Phorbol esters are between the diterpenes present to *Jatropha crucas* species and six types have been specific to *Jatropha curcas* [Haas *et al*., (2002)]. The intragastric LD50 to phorbol esters isolated of Jatropha *curcas* extract was spotted to be 27.34 mg kgG1 b.wt. In mice (believe highly toxic) and also that to histopathological alterations spotted were fatty vacuoles to the liver hepatocytes, overcrowded pulmonary alveolar capillaries and hemorrhage to the alveolus , abruption to cardiac muscle fibres glomerular atrophy and hemorrhage to the spleen[Li *et al*., (2010)]. These changes are coordinated to the spotted effects to our work and thus propositions that Jatropha extract with its contents must be acted singly or to combination by other toxic rules to the plant material to educed the severe pathological feedback. The exact mechanism to the spotted impact was not explain to the present study, however, it has been legalized that seed exert damage to tissues with the release to proteases, cytokines and also are activation to NADPH oxidase [Goel *et al*., (2007)].

3.7. Conclusion:

The present study has explain toxicity *Jatropha curcas* seed and ability to curb and inhibit liver cancer to mice and also that to careful use to the plant for classic treatment is hereby advised. additional give a wide berth is on-going of speculation the sub-chronic effects to low doses to the *Jatropha curcas* extract and also isolation to the specific toxic rules to *Jatropha curcas* seed so also that to raise to the highest possible level of therapeutic benefits.

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