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Impact of the Histological, Biochemical, and Cytogenetical Changes Caused by Ehrlich Solid Carcinoma (ESC) in the Pregnant Mice and Treated with Ethanolic Extract of *Tamarinds Indica* Seeds

Yasmin M. Abd El-Aziz[1,*], Mariam Y. Rashed[1], Donia W. Agamy[1], Hend M. Elmalaky[1], Rahma D. Abo-Elhamd[1], Mariam M. Elsergany[1], Rahma M. Abdelhay[1], and Ali H. Abu-Almaaty[1]

[1]Department of Zoology, Faculty of Science, Port Said University, Port Said, Egypt
***Corresponding author:** yasminabdelaziz2012@yahoo.com

ABSTRACT

All parts of *Tamarinds indica* plants are included in traditional medicine in India and other countries. There are many chemical values as phenolic materials that make it possess variable activities, most important of them antioxidant activity against cancer. The extract of *Tamarinds Indica* using to thwart various cancer types. Forty mice were divided into four groups; the first group was as control (normal mice), the second group was injected only with Ehrlich carcinoma cells, the third group was treated with *Tamarinds indica* extraction only, and the fourth group was injected with Ehrlich carcinoma cells and treated with *Tamarinds indica* extraction. At the final of the experiment, we represented the fourth group of mice has a significant reduction in cancer effects, which confirmed the anticancer activity of *Tamarinds indica* extraction *in vivo* (mice). Finally, the conclusion proves the efficacy of *Tamarinds indica* ethanolic extract as a cancer prophylactic chemotherapy in therapeutic approaches to treat cancer caused by Ehrlich cells in serum protein level, fetal tissues, cerebellum tissues, lung tissues, and chromosomes.

Keywords:
Ehrlich cells, *Tamarinds Indica*, Mouse, Histology, Cytogentics.

1. INTRODUCTION

The desired result of this study is to present an experiment in which cancer cells were exposed to mice (*in vivo*) and then treated with *Tamarind indica* seeds extract. The objective is to advance the creation process for facing the experimental malignancies in animals, particularly lab mice, which form the foundation of recent advances in cancer therapy [1]. Ehrlich ascites Carcinoma (EAC), which is considered a prevalent malignancy, which is extremely important for modeling. EAC is known also as a sundry carcinoma, which is initially hyperdiploid, highly transplantable, non-regressive, rapidly proliferating, short in life span, and 100% malignant. It also lacks transplantation antigen of tumor-specific (TATS), which prevents the host from rejecting tumor cells that carry the antigen because it doesn't stimulate the development of a cellular immune response. The liquid form is collected from the mouse's peritoneum, and because it contains both carcinoma cells and ascites liquid. At this stage,

using natural sources as an alternative cancer therapy is regarded to have considerable value for cancer regulation and the destruction of cancer programs because the perfect medicine has been focused on and found to be inefficient or minimally beneficial for normal cells [2].

Tamarinds indica is rich in protein and many needful amino acids that are necessary for human growth and development. They are also increasing in carbohydrates for energy sources, and minerals like potassium, phosphorus, calcium, and magnesium. Iron and vitamin A are both present in tamarind in little quantities. All tamarind parts are numerous employed for industrial and medical purposes. Thirty percent of the *Tamarinds indica* fruit pith is water. The commercially available arid tamarind pith contains 70 percent glucose and 30 percent fructose, along with 8 to 18 percent tanedioic tartaric acid and 25 to 45 percent reducing sugars. Pith has high carbohydrate and protein contents but little water [3]. The metabolites have a significant impact on their pharmacological effects. Alkaloids were found in the aqueous and alcoholic extracts after a phytochemical examination. *Tamarinds indica*'s most distinctive quality is its sweet, acidic flavor, which is primarily due to tartaric acid. Reducing sugars and acid (10%). A special plant acid called tartaric is composed of the primary once created, the photosynthetic products of carbohydrates are not used metabolically by the plant. Since tartaric acid concentration does not decrease as the fruit ripens, it is not being destroyed. used to promote fruit growth decreasing sugars increase to 22.30–40% throughout the same period of fruit growth, giving the acidic fruit a fuller flavor. The most prominent quality of *Tamarinds indica* fruit is that source of antioxidants, vitamin B, minerals, and sugar as well as being a good source of energy and antioxidants [4].

2. MATERIALS AND METHODS

2.1. Extraction of treatment:

Ethanol extract for *Tamarindus indica* seeds were obtained from the druggists at Port Said city, Egypt, which 200 mg of *Tamarindus indica* /Kg b.wt of mouse/ inject day after day for ten days, we collected and prepared the *Tamarindus indica* extract, washed the fresh seeds in the distilled water and left it to dried for 3 weeks. Then, gender these seeds by using a mortar (*Retsch*, Germany, RM 200), the powder dipping in 2/3 of 80% ethyl alcohol for three days. Then, it was filtered by using qualitative filter papers twice. Finally, evaporated the filtrated sample by using a rotary evaporator (*Stuart*, UK) at 50ºC and stored it in the vial at 5ºC for using (Figures 1 and 2).

Figure 1: *Tamarindus indica* seeds

Figure 2: Rotatory evaporator

2.2. Exposure of the mice to Ehrlich solid Tumor:

Ehrlich cells line was obtained from the National Cancer Institute (NCI) in Cairo, Egypt. $2.5*10^6$ tumor Ehrlich cells (TEC) were injected into muscle female mouse as one dose on the lower limb in the left femoral for starting our experiment after ten days from an injection [5].

2.3. Experimental animal and design:

The experiment was performed on forty pregnant Swiss mice, their weights were in the range of 25-27 gm, they were housed in plastic cages (ten per each cage), and we have equip 4 cages named: 1st group as control which injected with saline only for ten sequentially days, 2nd group as damage group which injected with EAC ($2.5*10^6$ tumor Ehrlich cells), 3rd group as treatment by ethanolic extraction of *Tamarindus indica* seed that injected by 200 mg/Kg b.wt for ten days, 4th group as treatment the AEC damage that doses as 2nd and 3rd groups. The cages were equipped with access to water and designed inside with plates suitable for eating. We have noticed them every day and they all are placed at room temperature. All adaptations were done for taking care of them and providing them with a suitable place, ventilation and humidity well. Balanced the mice day after day for growth rate determination and after the collected the samples, balanced the embryo, brain, and lung. According to Brenneman and Rigby [6], total protein parameter determination in serum. Histopathological studies for embryo, adult' cerebellum, and adult lung [7,8]. And a cytogenetical study [9], mice have 20 pair of chromosomes, after using damage with EAC there are many chromosomal aberrations appears like deletion, chromosomal break, fragmentation, and ring chromosomes. According to Ludbrook [10], the statistical analysis was done to compare the parameters and performed the differencing significantly as $p \leq 0.05$, the statistical data was calculated as mean±SEM by using the SPSS.16 program.

3. RESULTS AND DISCUSSION

3.1. Growth rate: Weight gain calculation for a body in the four groups, with the time the weights of 1st and 3rd groups increased. Decreased the gain weight in 2nd group. In group 4th, the weight gain decreased as compared with 1st and 3rd groups but increased as compared with 2nd group (Figure 3 and table 1).

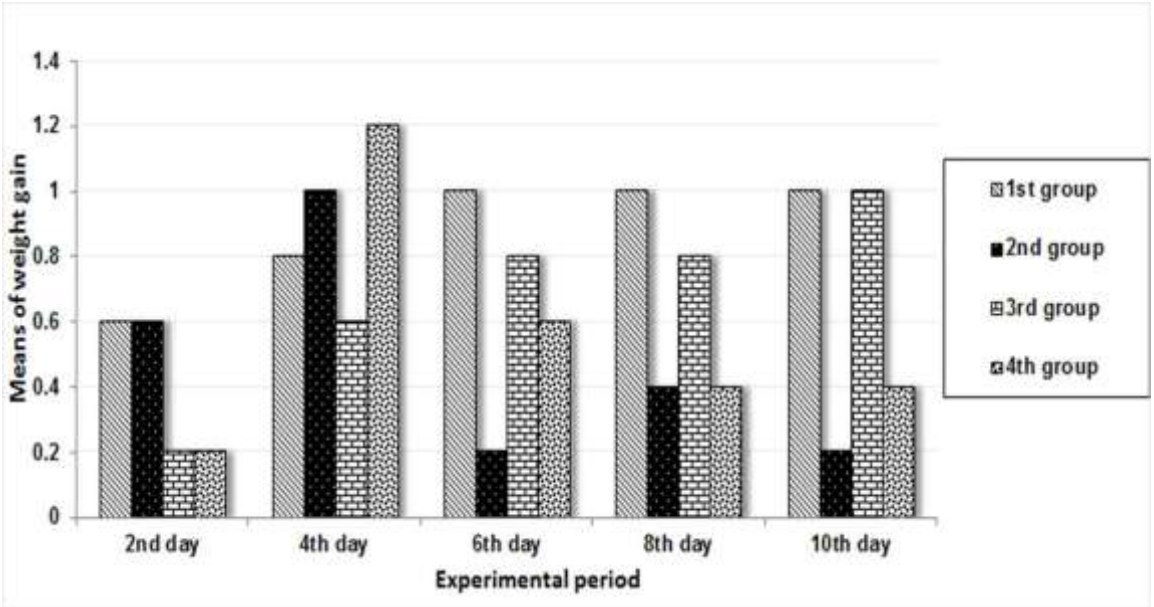

Figure 3: Chart showing the weight gain for a body in the four experimental groups.

Table 1: The weight gain for a body of the four experimental groups in the experimental period.

	2nd day	4th day	6th day	8th day	10th day
1st group	0.6	0.8	1	1	1
2nd group	0.6	1	0.2	0.4	0.2
3rd group	0.2	0.6	0.8	0.8	1
4th group	0.2	1.2	0.6	0.4	0.4

3.2. Embryos' weights and lengths: The means of absolute weights (g) and lengths (cm) for embryos were represented in figure 4 and table 2 as mean±SEM for four experimental groups. The

normal means for embryos' weights are 1.043±0.087, in 2nd group were noticed decrease of the embryos' weights to 0.528±0.022, the 3rd group showed nearly similar to 1st group were 1.011±0.072, while, induction of the *Tamarindus indica* extraction (4th group) increased the embryos' weights are 0.599±0.038 that compared to 2nd group. The length means for mice embryos, in the 2nd group decreased compared with the 1st group from 3.03±0.127 to 2.37±0.103, the means embryos' lengths in 3rd group are 2.90±0.098 near to 1st group. by induced treatment of the extraction, after being exposed to EAC mice, the means of embryos' lengths are increased to 2.75±0.122 as compared with the 2nd group (damage group).

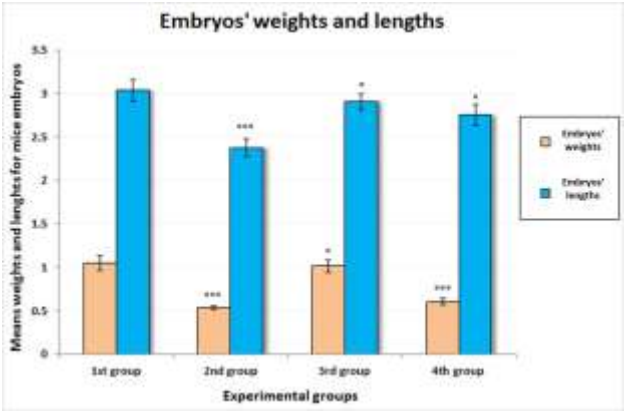

Figure 4: Chart representing the mean±SEM for the embryos' weight and lengths of the four experimental groups. (*) non-significant → p>0.05, and (***) highly significant → p≤0.01 that compared with 1st group (negative control).

Table 2: The parameters (weights and lengths) for embryo mice in the four experimental groups.

Parameters \ Experimental groups	1st group	2nd group	3rd group	4th group
Embryos' weights	1.04±0.09	0.53±0.02***	1.01±0.07*	0.60±0.04***
Embryos' lengths	3.03±0.13	2.37±0.10***	2.90±0.10*	2.75±0.12*

3.3. Adult brain and lung weights: The means of absolute weights (g) for adult brain and adult lung were represented in figure 5 and table 3 as mean±SEM for four experimental groups. The normal means for the brain are 0.253±0.037, in the 2nd group noticed increased the brain weights to 0.382±0.012, the 3rd group was showed nearly similar to 1st group were 0.274±0.031, while, induction of the *Tamarindus indica* extraction (4th group) decreased the brain' weights are 0.340±0.020 that compared to 2nd group. A Zimber and WJ Visek discovered in 1972 that mice administered 12x 10^6 EAT tumor cells later developed tumors at three weeks that presented 40 percent in weight of the mouse's brain weight [11]. Dysentery and persistent diarrhea are both treated by tamarind seed extract. For treating ulcers, boils, rashes, eye and skin inflammation, and dyspepsia, and to speed up the healing of wounds, the roots and bark are employed. Swollen joints, boils, and sprains can also be treated with the plant's leaves and blooms. The plant has been role as anti-inflammatory, antihelminthic, antioxidant, hepatoprotective, cytotoxic, antibacterial, analgesic, antiasthmatic, hypolipidemic, and weight-reducing properties [12]. However, the lung weights mean for adult mice, in the 2nd group increased compared with the 1st group from 0.166±0.011 to 0.249±0.030, the means of lung weights in the 3rd group are 0.162±0.007 which is like to 1st group. By induced treatment of the extraction, after being exposed to EAC mice, the means of lung weights are decreased to 0.204±0.013 as compared with the 2nd group (damage group). The relative lung weight significantly decreases in the Ehrlich ascites carcinoma model [13], and there were no discernible alterations in the relative lung weight, according to research on tamarind water extract [14].

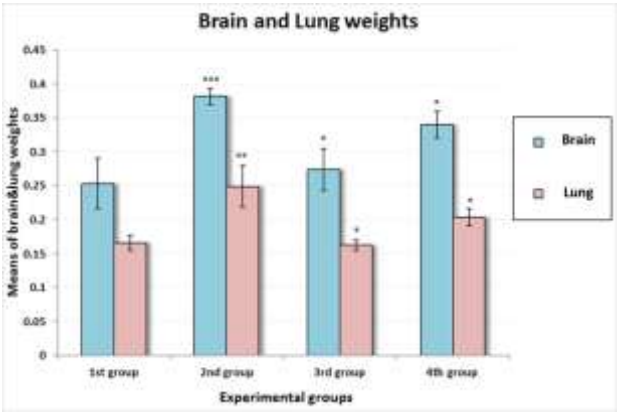

Figure 5: Chart exhibiting the mean±SEM of the adult brain and lung weights for the four experimental groups. (*) non-significant → p>0.05, (**) significant → p≤0.05, and (***) highly significant → p≤0.01 that compared with 1st group (negative control).

Table 3: The parameters (brain and lung) for adult mice in the four experimental groups.

Parameters \ Experimental groups	1st group	2nd group	3rd group	4th group
Adult' brain	0.25±0.04	0.38±0.01***	0.27±0.03*	0.34±0.02*
Adult' lung	0.17±0.01	0.25±0.03**	0.16±0.01*	0.20±0.01*

3.4. Level of total protein in serum: The means of total protein contents (g/dL) in serum of adult mice showed increased means level when compared with 1st group, and the induced ethanolic extraction of seeds the *Tamarindus indica* to malignant mice, the enhancement mean levels of total protein that compared to 2nd group that increased from 5.71±0.298 to 6.53±0.401 and recorded in figure 6 and table 4 as mean±SEM for four experimental groups. In other experiments to detect disorders in the protein serum with transplanted Ehrlich carcinoma, the outcomes recognize new proteins during cancer growth, 16 of them are distinguished in protein gel electrophoresis. Cellular proteins are apoptosis mediating proteins, transcription, structural and transport proteins, DNA reparation, and replication factors, which are fundamental for mitosis. It is believed that the expansion in their content in mice with cancer is because of the expansion in several dividing cells [15-18].

In the 4th group (treatment after injury group), the means of total protein contents in serum are 5.71±0.298 which is down-regulated as compared to the damage group (2nd group). *T.indica*, as it knows, is utilized traditionally as a cure, and every part of it (fruit, leaves, and seeds which are embedded in a pith) has other uses like chemical materials, nutritional use, drug compounds, textile industries, and may utilize as wood, mash, and fuel [19].

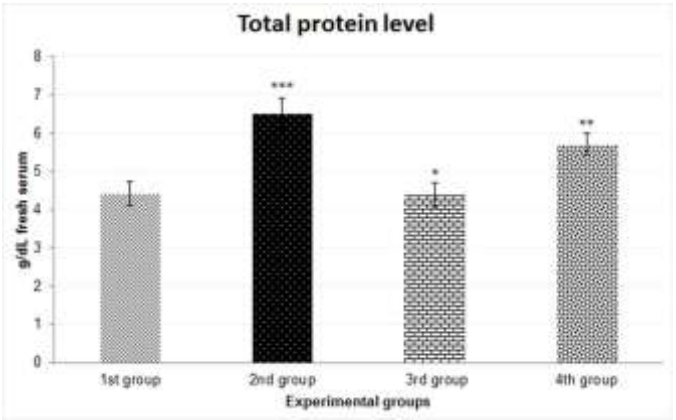

Figure 6: Chart showing the mean±SEM for the level of total protein in mice's serum of the four experimental groups. (*) non-significant → p>0.05, (**) significant → p≤0.05, and (***) highly significant → p≤0.01 that compared with 1st group (negative control).

Table 4: The parameters (brain and lung) for adult mice in the four experimental groups.

Parameter / Experimental period	1st group	2nd group	3rd group	4th group
Total protein level	4.42±0.32	6.53±0.40***	4.39±0.32*	5.71±0.30**

3.5. Histological examination for the embryo, cerebellum, and lung: The embryo mice micrograph of the normal control negative group (1st group) which stain with hematoxylin and eosin (H&E) showed normal structure for the appearance of the organs tissues in mice embryo as the normal epithelium of olfactory, salivary gland, brain, backbone, lung, heart with homogeneous of atrium blood, small intestine, kidney, and lower limb, that representing in figure 7a. After induction of the pregnant mice to $2.5*10^6$ of EAC (2nd group), the malignant cells affected the embryo disorders as the transmigration of malignant cells in olfactory epithelial tissues, draining blood atrium of heart which engorged with blood, segmentation of brain, the necrotic portion was appeared in neck zone of backbone, the large size of the kidney, and damage morphology in the lower limb that showing in figure 7b. While the mouse embryo from the mouse injected with 200 mg of *Tamarindus indica* extraction/Kg b.wt (3rd group) represented the normal histological structures as in figure 7c that liked mouse embryo from the control negative group (1st group). A subcutaneous ascites of the Ehrlich tumor cause atrophy, fetal gene activation, and cardiac fibrosis. Mice with subcutaneous EAC tumors develop cardiomyopathy [20]. The exposure of EAC to mice, defects in their fetus are shortness in size, its head is flat and curved, the embryos' palate is cleft in small parts, and also, the thickness of the epithelial tissues in the core of chondro-structure [21-23].

When induction *Tamarindus indica* extraction to malignant mice (4th group), the mouse embryo showed mild disorder of the histological morphology as increased the lumen of the olfactory canal, limited normal epithelium in the olfactory zone, decreased the blood atrium of the heart, and enhanced size of kidney and other organs that representing in figure 7d.

The cerebellum tissue layers of adult mice form four groups representing the normal structure of epithelium and loose connective tissues in the control negative group (1st group) as displayed in figures 8a and 8b; normal structure of cerebellum three layers, molecular layer, Purkinje cell layer, the granular layer, and white matter. Also, the normal structure of the cerebellum tissues was liked a negative control in *the Tamarindus indica* extract induced group (3rd group) that is exhibited in figure 8f. The cerebellum tissues from the 2nd group (damage group) appeared in stages of necrosis as pyknotic nuclei in some Purkinje cells and the large portion between the Purkinje and granular layers as shown in figures 8c, 8d, and 8e. But, the micrograph of cerebellum tissues from mice in the 4th group showed ameliorative tissues as the perfection of the Purkinje cells in the Purkinje cell layer, the granular layer, molecular layer, white matter, and decreased the edema zone between the Purkinje and granular layers as presented in figure 8g and 8h.

In tissues of lung mice from four groups showing the normal structure of tissues for lung (1st group) representing normal alveoli, ducts of alveolar, blood vessels, and bronchioles that exhibited in figures 9a and 9b. Occurred the tissue defects in epithelial and loose connective of lung tissues in 2nd group caused the migration of malignant tumor cells to lung tissues represented as blood vessels were congestion with blood, leakage of inflammatory cells within areolar connective tissues of the lung, fibrous within areolar connective tissues of the lung, thickness the endothelium which around the blood vessels, formed fibrous layer, alveoli, ducts of alveolar, and bronchioles that showed in figures 9c, 9d, and 8e. Also, appeared the normal structure of lung mice tissues from the 3rd group showed that alveoli, ducts of alveolar, blood capillaries, and bronchioles as presented in figures 9f and 9g. After treatment by inducing the *Tamarindus indica* extraction to malignant mice (4th group) represented that some blood vessels were congested, decreased the inflammatory cells leakage around bronchioles and

inside blood vessels which thickness the endothelium as fibrous formation around blood vessels in lung tissues, alveoli, and ducts of alveolar that showed in figure 9h, 9i, and 9l.

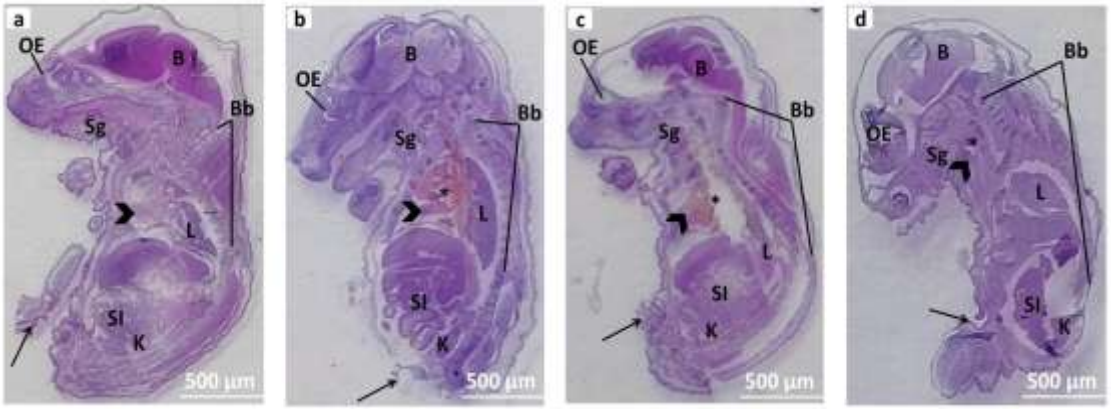

Figure 7: Longitudinal sagittal micrograph of embryo mice for four groups was showing; **a)** Normal structure from 1st group; normal epithelium of olfactory (OE), salivary gland (Sg), brain (B), backbone (Bb), lung (L), heart (arrowhead) with atrium blood, small intestine (SI), kidney (K), and lower limb (arrow). **b)** In the 2nd group, the tissue defects representing the transmigration of malignant cells in olfactory epithelial tissues (OE), draining blood atrium of the heart (asterisk) which engorged with blood, segmentation of brain (B), the necrotic portion was appeared in neck zone of the backbone (Bb), the large size of the kidney (K), and damage morphology in the lower limb (arrow). **c)** Mice embryo from 3rd group represented the normal histological structures like mice embryo from 1st group. **d)** Mice embryo from the 4th group showed mild disorder of the histological morphology as increased the lumen of the olfactory canal, limited normal epithelium in the olfactory zone (OE), decreased the blood atrium of the heart, and enhanced size of kidney (H&E, 500 µm).

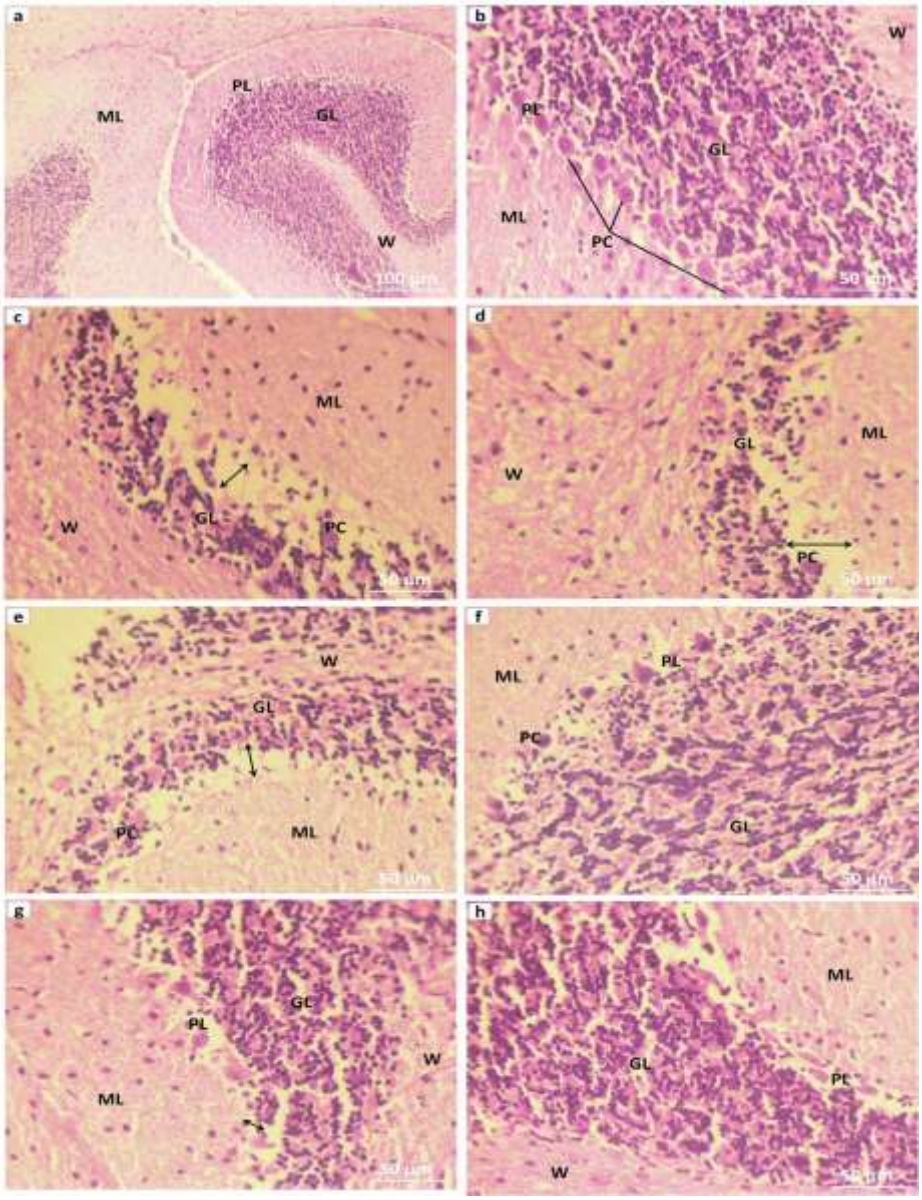

Figure 8: Cerebellum sections micrograph of adult mice for four groups was represented as in **a and b)** Normal structure from 1st group; normal architectural of cerebellum layers, molecular layer (ML), Purkinje cells (PC), Purkinje cell layer (PL), the granular layer (GL), and white matter (W). **c, d, and e)** Cerebellum tissues from mice of the 2nd group, pyknotic in some Purkinje cells (PC) on the Purkinje cell layer (PL), and the large portion between the Purkinje (PL) and granular (GL) layers, and white matter (W). **f)** Micrograph of cerebellum tissues from mice of 3rd group showed the normal structure of layers that similar to 1st group. **g and h)** Ameliorative of cerebellum tissues appeared in the 4th group as the perfection of the Purkinje cells (PC) in the Purkinje cell layer (PL), the granular layer (GL), molecular layer (ML), white matter (W), and decreased the edema zones ( ) between the Purkinje (PL) and granular (GL) layers (H&E, a→100 µm, and b, c, d, e, f, and g →50 µm).

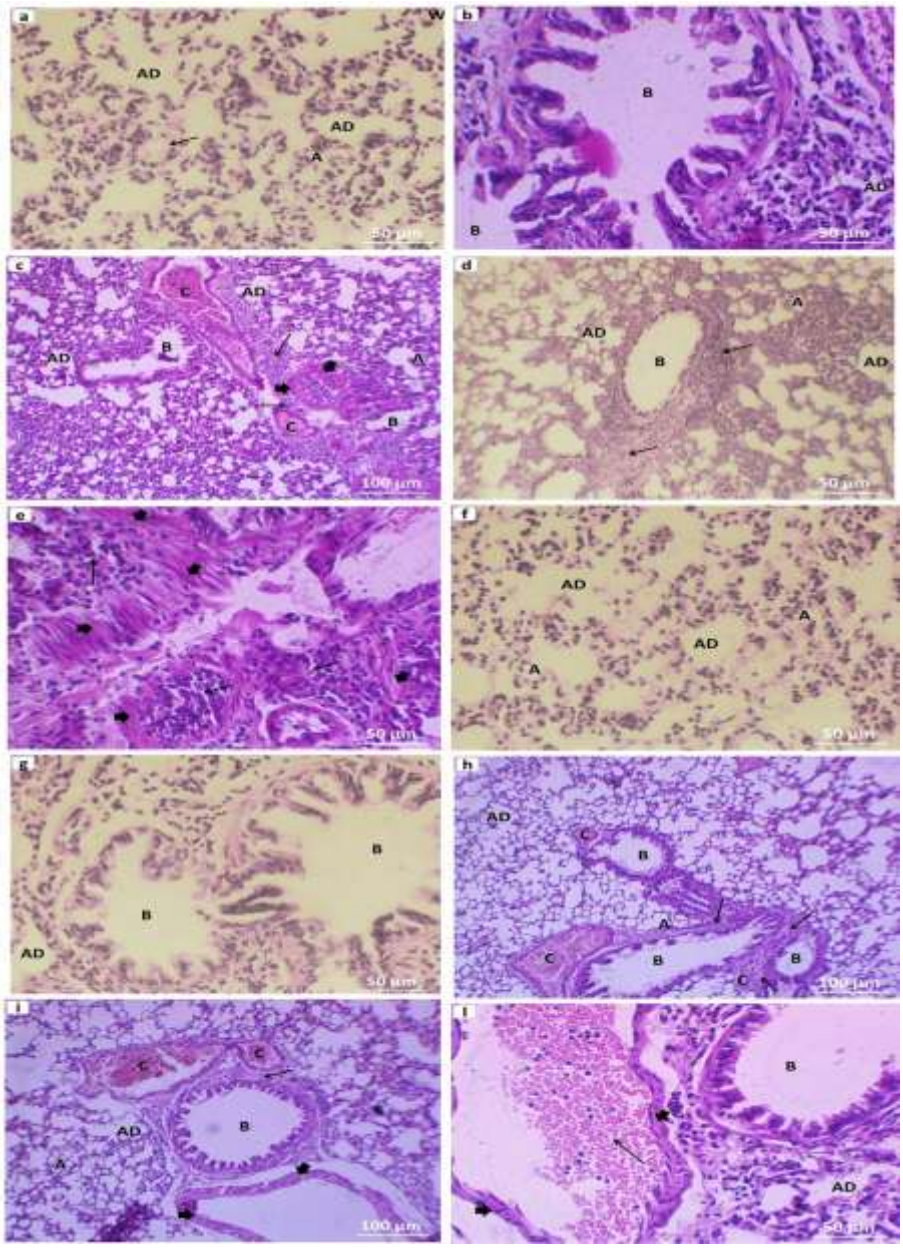

Figure 9: Cross micrograph adult lung section from four groups showing as **a and b)** Normal appearance tissues for lung from 1st group representing in normal alveoli (A), ducts of alveolar (AD), blood vessels (thin arrows), and bronchioles (B). **c, d, and e)** Changes histological in lung epithelial and loose connective tissues in 2nd group caused migration of malignant tumor cells to lung tissues represented as blood vessels were congestion (C) with blood, leakage of inflammatory cells (thin arrows) within areolar connective tissues of the lung, fibrous (thick arrows) within areolar connective tissues of the lung, thickness the endothelium which around the blood vessels, formed fibrous (thick arrows) layer, alveoli (A), ducts of alveolar (AD), and bronchioles (B). **f and g)** Normal structure tissues for lungs from mice of the 3rd group showed alveoli (A), ducts of alveolar (AD), blood capillaries, and bronchioles (B). **h, i, and l)** In mice' lungs from the 4th group represented that some blood vessels were congestion (C), decreased inflammatory cells (thin arrows) leakage which around bronchioles (B), and inside blood vessels which thickness of the endothelium as fibrous (thick arrows) formation around blood vessels in lung tissues, alveoli (A), and ducts of alveolar (AD) (H&E, c, d, h, and i →100 µm and a, b, e, f, g and l → 50 µm).

3.6. Examination of chromosomal aberration:

In the present, our experiment, study the disorder in chromosomes and compared it with normal chromosomes as presented in figure 10. The normal 40 chromosomes in the 1st and 3rd groups that

shown in figures 10a and 10f. Chromosomal changes such as break, deletion, fragmentation, iso-chromatid fragmentation, and ring chromosomes from mice in the 2nd group that exhibited in Figures 10b, 10d, 10c, and 10e. Tumor ascites exposed to the mice effect in chromosomes showed a completely wide variation of their numbers, from 50 to 84, a large telocentric chromosome with a prominent secondary constriction, and the large metacentric chromosome with about equal arms, min chromosomes, smaller than any normal mouse chromosomes was in case of chromosomal structure [24].

Aberrations of chromosomes have decreased after induced *Tamarindus indica* extraction to malignant mice (4th group) represented as break, deletion, and fragmentation (Figures 10g and 10h). According to Aravind *et al.* [24] using the polysaccharide extracted from the *Tamarindus indica* seeds kernel (PST001) for the treatment of Daltons ascites lymphoma and Ehrlich ascites carcinoma, has apoptotic properties and its proliferation inhibition as the cell morphology analysis showing nuclear shrinkage, fragmentation of the cells, chromatin condensation and apoptotic bodies in PST001 treated cells in contrast to control cells. The fragmentation of chromosomal DNA is a hallmark of apoptosis and may facilitate apoptosis by terminating DNA replication and gene transcription. The degradation of DNA down to oligonucleosomal fragments is a late event of apoptosis. This confirmed our study in the use of *Tamarinds indica* ethanolic extraction for the treatment of the damage effects that occurred.

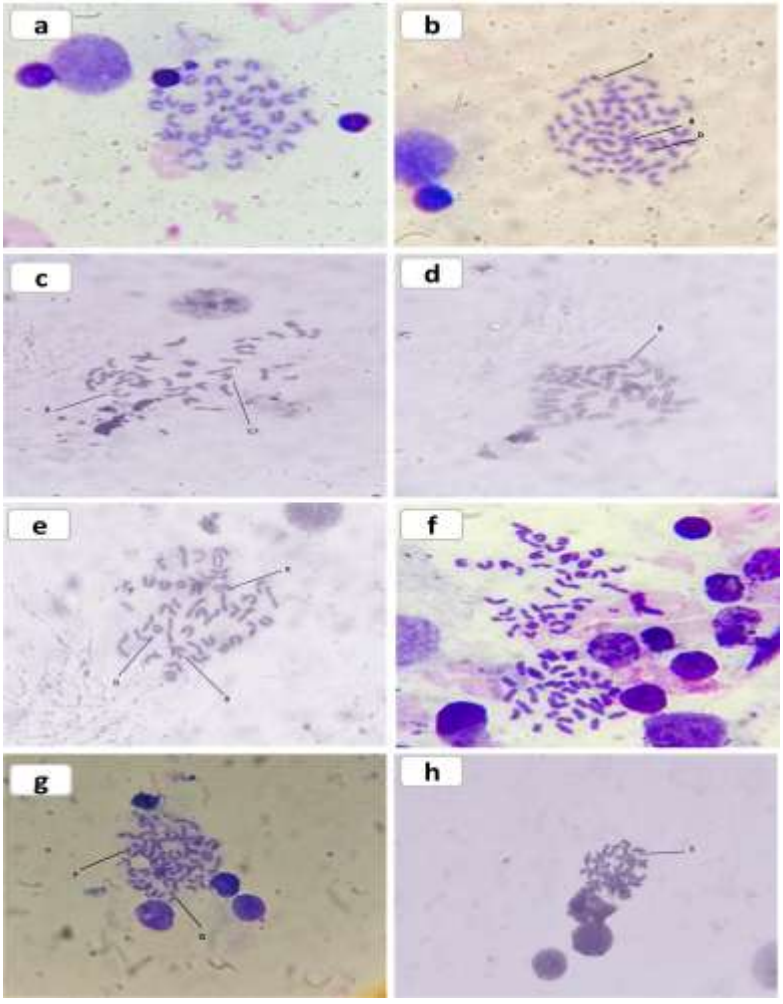

Figure 10: Bone marrow smears for examination of the cytogenetical chromosomes of mice in four groups represented as **a and f)** Normal 20 pairs of chromosomes from 1st group as control negative group and 3rd group as normal 40 chromosomes. **b, c, d, and e)** Chromosomal disorders from the 2nd group showed as a break (B), deletion (D), fragmentation (F), iso-chromatid fragmentation (ICF),

and ring chromosomes (R). **g and h)** Chromosomal changes from the 4th group were represented as a break (B), deletion (D), and fragmentation (F) (Giemsa, 1000X).

4. CONCLUSION

The conclusion in the present experiment proves the usefulness of the *Tamarinds indica* ethanolic extraction for treating the effect of cancer caused by Ehrlich cells.

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