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Embryonic Development of Blowfly Inducing Myiasis, *Chrysomya Albiceps* Affected by Gamma Radiation

Ahmed M.A. Elnaggar[1], Ahmed S. Bream[1*], Ahmed Z.I. Shehata[1], Ahlam Gabarty[2]
1- Department of Zoology, Faculty of Science (Boys), Al-Azhar University, Nasr City, Cairo, Egypt.
2- Natural Products Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt.
Corresponding author: Ahmed S. Bream, **E-mail address:** ahmedbream60@azhar.edu.eg, **Mobile Phone:** (+20)01123455965

ABSTRACT
Background: *Chrysomya albiceps* has a great medical and sanitary importance, causing infestation of human by the larvae (myiasis). Also, *C. albiceps* mechanically can transmit bacteria, viruses and helminths to human and animals.
Materials and Methods: larvae of *C. albiceps* were collected using fish as media. The irradiation process carried out using Gamma cell-40 (cesium-137 irradiation unit) at the rate of 2.3 Gy/min (doses: 4 and 10 Gy). Eggs laid by females resulted from irradiated pupae were taken and subjected to embryogenesis examination at different intervals of time (every hour from 1 to 13 hours).
Results: First cleavage nuclei were observed after 1 hour from oviposition, after about 2 and 3 hours, the cleavage nuclei became surrounded by a halo of cytoplasm and migrated toward the periphery of the egg. After 4 hours, the cleavage cells were reached the periphery of egg leaving few nuclei in the center which form the vitellophages, almost all cleavage cells were merged within the periplasm to form continuous cellular layer or the "blastoderm" after five hours from oviposition. The gastrulation was appeared as furrows in the germ band within 8 hours from oviposition. Complete gastrulation was attained within 8-10 hours. At 4 Gy of gamma radiation, blastoderm was formed within 7 hours and gastrulation was attained within 12 hours from oviposition. Organogenesis continued normally, foregut and hindgut were formed within 13 hours. **Conclusion:** lower dose of gamma radiation (4 Gy) induced a prolongation in the embryogenesis as compared with control, while 10 Gy of gamma radiation inhibited the embryogenesis by blocking the gastrulation.
Keywords: *Chrysomya albiceps,* Embryonic development, gamma radiation, gastrulation.

INTRODUCTION
The blowfly, *Chrysomya albiceps* (Family: Calliphoridae) is one of the most important blowflies studied, causing an infestation of human and animal body tissues by fly larvae [1, 2, 3].

Chrysomya albiceps widely distributed in Africa and Egypt throughout different governorates particularly in fish markets [1, 3] and can readily sneak inside hospitals, especially those that contain large numbers of patients with diabetic foot ulcers. Also, *C. albiceps* is one of the most blowflies recognized as a major species in the colonization of carcasses and corpses and mechanically can transmit bacteria, viruses and helminths to human and animals [1, 2, 4, 5].

Gamma radiation used in Sterile Insect Technique (SIT) as a non-disruptive and species-specific vector control strategy to reduce vector numbers and then eliminate the spread of diseases by affecting reproduction, fecundity, fertility, and embryonic development [6, 7]. On the other hand, the embryonic development and growth period is the most vulnerable phase in insects' life in which a highly refined biochemical and biological processes occurs in a definite time to organize required molecular, cellular and tissue mechanisms until hatching [8]. Many factors, including chemical and physical agents, mental stress, psychological trauma, and several others, possess a disturbing effect on normal patterns of biochemical and biological mechanisms occurring during embryogenesis. A prolix report on the effects of gamma radiation on embryonic development of insects is not available.

AIM OF STUDY
The present study performed to investigate the effect of different doses of gamma radiation on the embryonic development of the blowfly, *C. albiceps.*

MATERIALS AND METHODS
Chrysomya albiceps culture:
Larvae of *C. albiceps* were collected around Animal House, Faculty of Science (boys), Al-Azhar University, Cairo, Egypt using fish as media. Collected larvae were transmitted to Medical Entomology insectary, Animal House, Al-Azhar University, Cairo, Egypt and reared for six generations using standard rearing described procedure [9].

Irradiation process:
The irradiation process carried out using Gamma cell-40 (cesium-137 irradiation unit), National Center for Radiation Research and Technology (NCRRT), Cairo. The dose rate was 2.3 Gy/min at the time of the present investigation.

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Preparation of eggs for the embryogenesis study:

To study the histogenesis and differentiation of the embryonic development of *C. albiceps*, pupae were exposed to gamma radiation at the doses of 4 and 10 Gy. Females resulted from treated pupae were isolated with normal males obtained from the colony and transferred into wooden cages containing cotton pads soaked in 10% sucrose solution and milk powder. Females allowed to lay eggs on fresh liver. Newly laid eggs (immediately after oviposition) were divided into 17 groups (each of 20 eggs). Each group was placed on a moistened piece of cotton in a pteri-dish and kept at 27±2°C and RH of 70-75%. Five eggs were taken at different intervals of time (every hour from 1 to 13 hours).

From each group, eggs were dechorionated by placing them in 3.0% sodium hypochlorite solution for 5 min. or until the egg become transparent and then rinsed several times in distilled water. The eggs were fixed in Bouin's solution for two min. and washed thoroughly in 50.0% ethyl alcohol. Later, eggs were embedded in paraffin, serially sectioned at 6 µ, stained with Delafield's haematoxylin, dehydrated, cleared in xylol and then mounted in Canada balsam.

Statistical analysis

Results were analyzed using variance analysis (ANOVA) [10]. Data were coded and entered using the statistical package SPSS V.22. A probability value (P value) less than 0.05 was considered statistically significant. Data visualization becomes available using R-studio V.4.1.3.

Ethical Approve: The study followed the ethical customs of Faculty of Science, Al-Azhar University.

RESULTS

1. Untreated eggs (Control):

Chrysomya albiceps eggs elliptical, translucent, and pearly white (Figure 1 a). Anteriorly, the egg is taper than its' blunt posterior end. The egg body covered by a chorion. Figure 1 (b,c), clearly revealed that the cytoplasm is forming a bounding layer, the periplasm and an irregular reticulum within the yolk. In fertilized egg, the nucleus of zygote usually located close to the egg periphery, where it submits to division. After division, the polar bodies (cleavage nuclei) are formed (Figure 1 d,e).

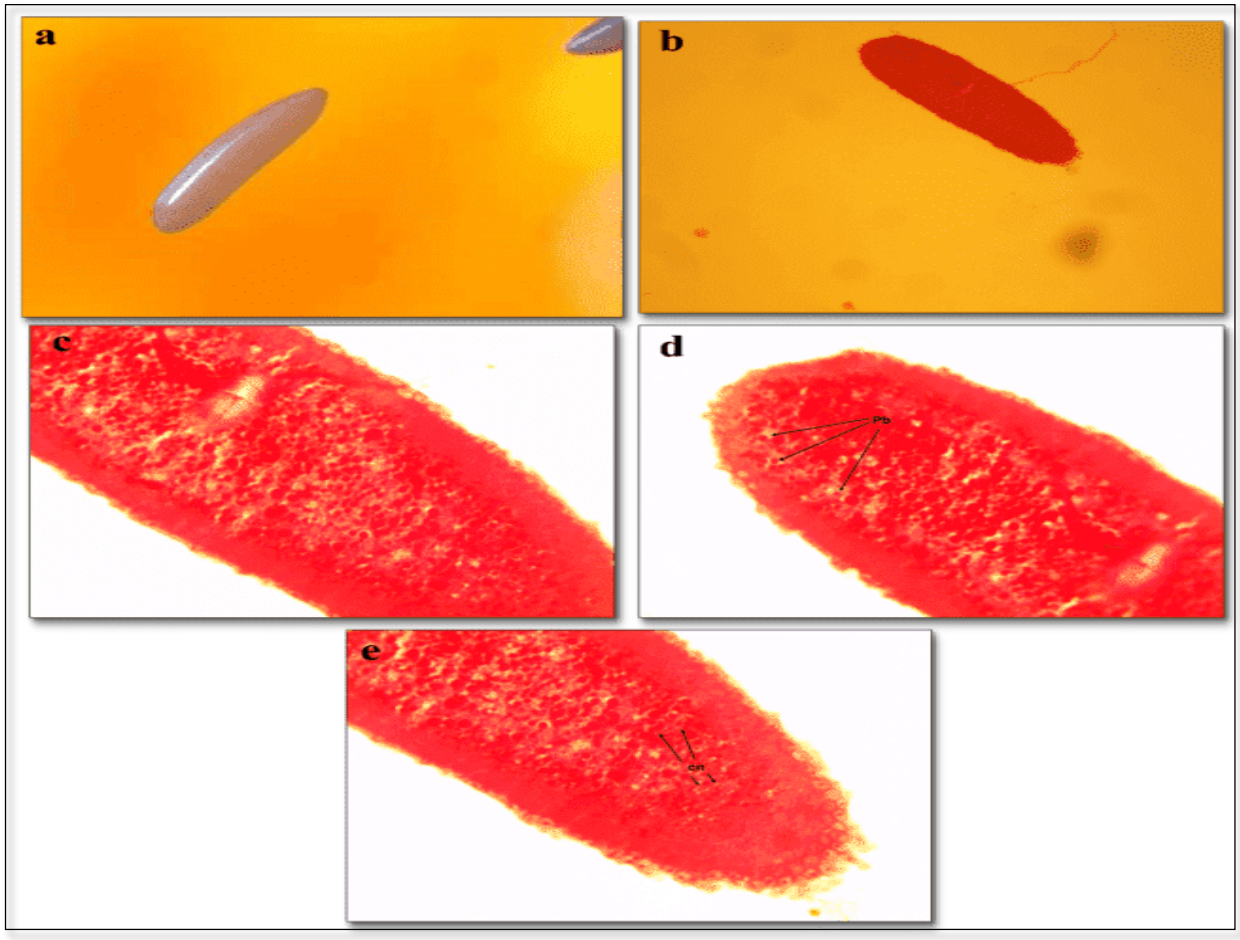

Figure 1. (a-e): *Chrysomya albiceps* untreated egg, (a) *C. albiceps* egg at 100 X, (b) Sagittal section in the untreated egg of *C. albiceps* 5 min. after oviposition at 100 X, (c) Sagittal section in the untreated egg of *C. albiceps* 5 min. after oviposition at 400 X, (d) Sagittal section in untreated egg of *C. albiceps* showing polar bodies (Pb) at 400 X and (e) Sagittal section in untreated egg of *C. albiceps* showing cleavage nuclei (cn) at 400 X.

1.1. Histogenesis and differentiation:

The histological studies of *C. albiceps* embryogenesis dealt with the early two major stages (blastoderm formation and gastrulation).

1.2. Cleavage and blastoderm formation:

After 1 hr. from oviposition, the first cleavage nuclei were observed after 1 hour from oviposition, by a superficial division in eggs; in which a large mass of centrally located yolk confines cleavage to the cytoplasmic rim of the egg and vacuoles were formed in the cytoplasm (Figure 2 a,b). After about 2 and 3 hours, the cleavage nuclei were decreased in the egg center but evenly distributed towards the periphery, where the mitoses continue but at a progressively slower rate.

During the ninth division, about five nuclei reached to the surface of posterior pole of the embryo, the nuclei become enclosed by cell membranes and formed the polar cells that give rise to the gametes of the adult. Each nucleus was surrounded by a halo of cytoplasm (Figure 2 c). In addition, the vaculation of the cytoplasm in the interior of the egg was evidently increased (Figure 2 d).

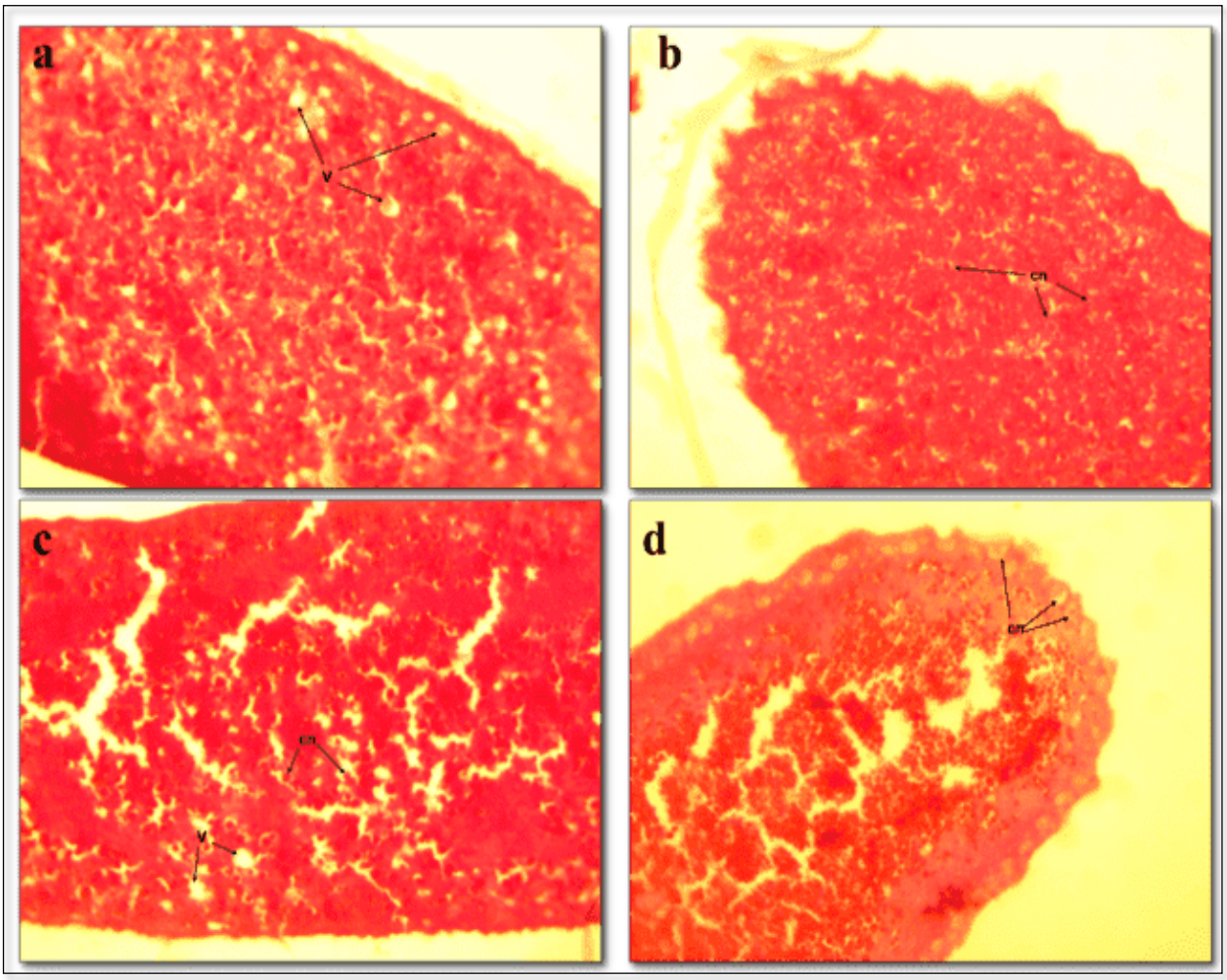

Figure 2. (a-d): (a) Sagittal section in untreated egg of *C. albiceps* 1 hr. after oviposition showing vacuoles (v) at 400 X, (b) Sagittal section in untreated egg of *C. albiceps* 2 hrs. after oviposition showing cleavage nuclei (cn) at 400 X, (c) Sagittal section in untreated egg of *C. albiceps* 3 hrs. after oviposition showing cleavage nuclei (cn) surrounded by a hallo of cytoplasm and vacuoles (v) at 400 X and (d) Sagittal section in untreated egg of *C. albiceps* 4 hrs. after oviposition showing cleavage nuclei (cn) toward the periphery at 400 X.

After 4 hours, the cleavage nuclei were almost absent from the center but evenly reached the periphery leaving few nuclei, in addition a great number of vacuoles in the cytoplasm appeared (Figure 3 a), the remaining nuclei in the center form "primary vitellophages". Within five hrs. from oviposition, almost all cleavage nuclei were merged within the periplasm to form a continuous cellular layer or the (blastoderm) surrounding the yolk in which all the cells are arranged in a single-

layered jacket around the yolky core of the egg (Figure 3 b). As soon as the cellular blastoderm is completed and a thick layer of blastoderm formed represents the "germ band" which must be developed into the future embryo (Figure 3 c).

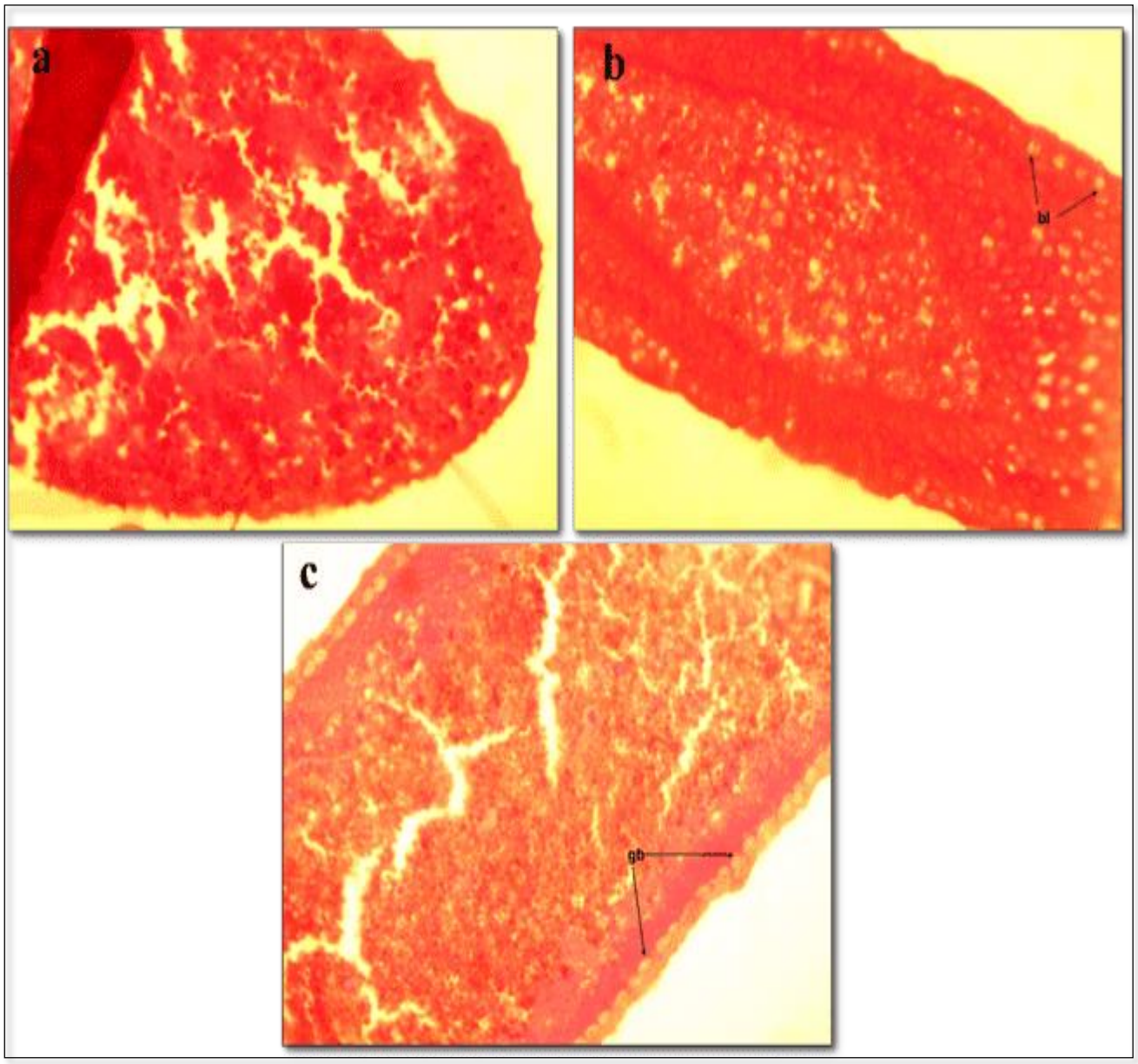

Figure 3. (a-c): (a) Sagittal section in untreated egg of *C. albiceps* 4 hr. after oviposition 400 X, (b) Sagittal section in untreated egg of *C. albiceps* 5 hrs. after oviposition showing blastoderm (bl) at 400 X and (c) Sagittal section in untreated egg of *C. albiceps*, 6 hrs. after oviposition showing germ band (gb) at 400 X.

1.3. Gastrulation:

Gastrulation is the process by which the mesoderm and endoderm are invaginated within the ectoderm (germ band). Within 7 hours from oviposition a ventral furrow initiating gastrulation was formed (Figure 4 a). At 8 hours from oviposition, also an anterior or cephalic furrow across the anterior third of egg and another one posterior furrow were formed (Figure 4 b,c,d). The complete gastrulation was attained within 8-10 hours (Figure 5 a,b).

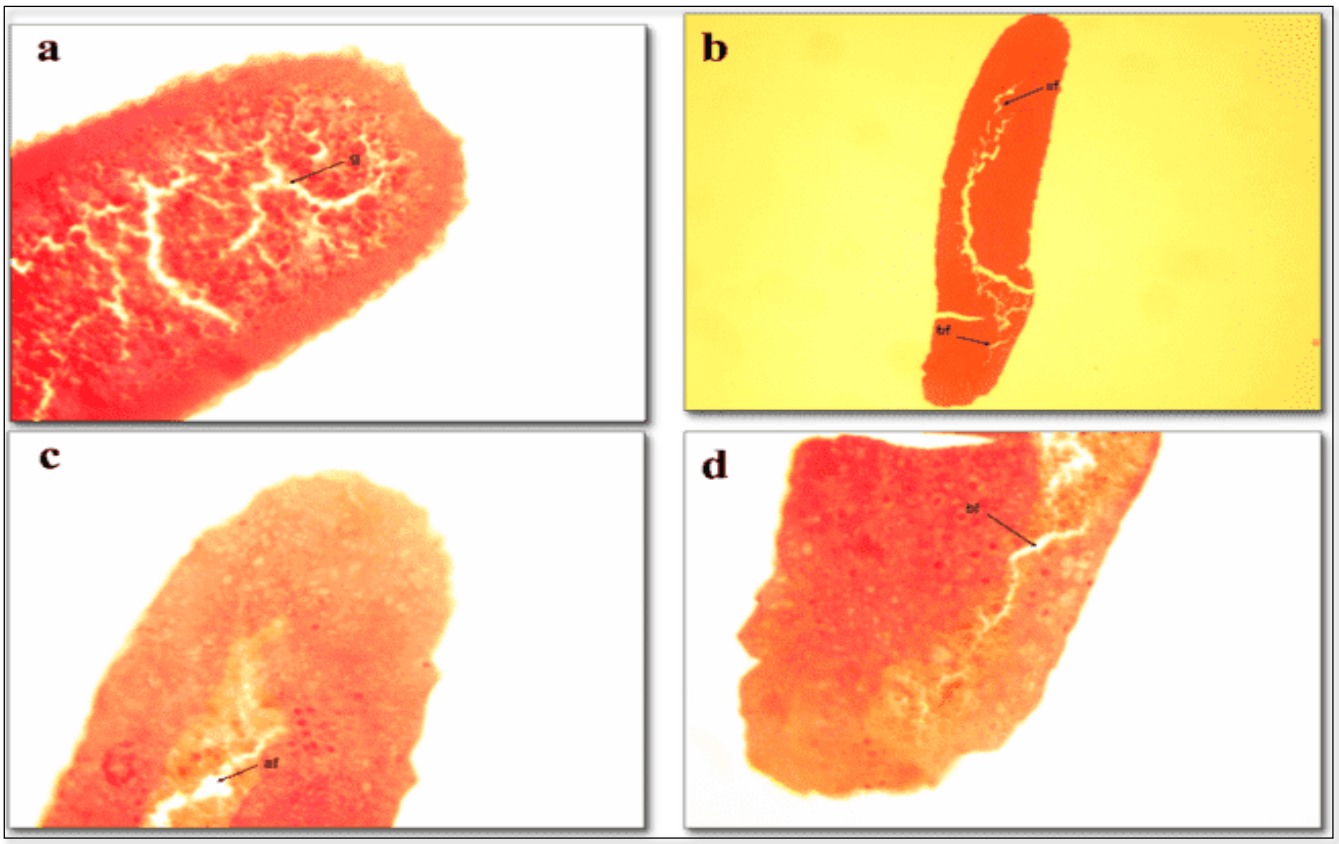

Figure 4. (a-d): (a) Sagittal section in untreated egg of *C. albiceps* 7 hrs. after oviposition showing gastrulation (g) as a furrow at 400 X, (b) Sagittal section in untreated egg of *C. albiceps* 8 hrs. after oviposition, showing the anterior and posterior furrows (af), (pf) at 100 X, (c) Sagittal section in untreated egg of *C. albiceps* 8 hrs. after oviposition showing the anterior furrow (af) at 400 X and (d) Sagittal section in untreated egg of *C. albiceps* 8 hrs. after oviposition showing the posterior furrow (pf) at 400 X.

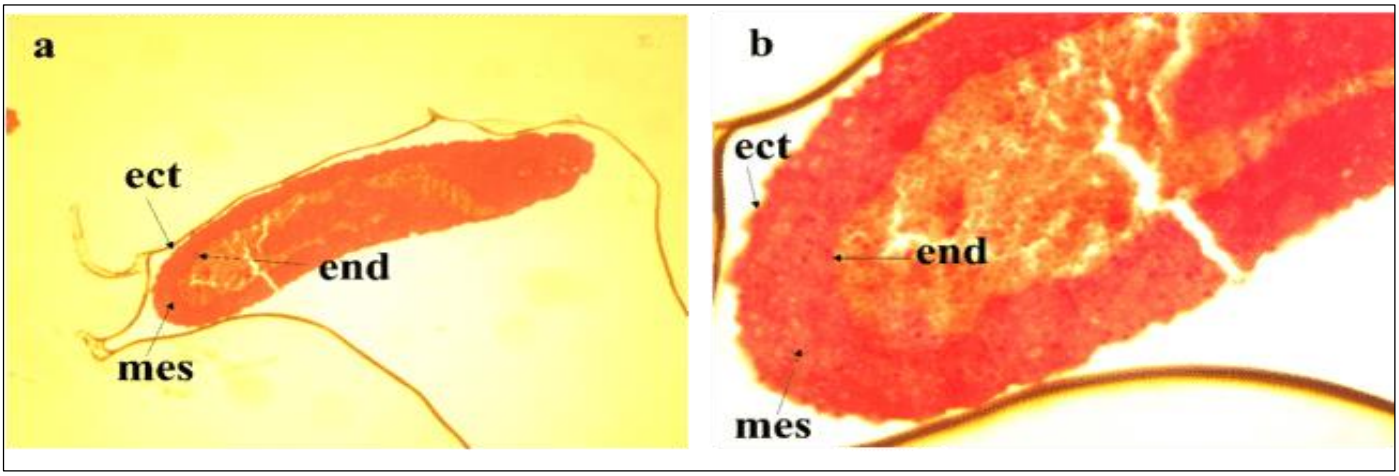

Figure 5. (a,b): (a) Sagittal section in untreated egg of *C. albiceps* 8-10 hrs. after oviposition showing complete gastrulation ectoderm (ect), endoderm (end) and mesoderm (mes) at 100 X and (b) Sagittal section in untreated egg of *C. albiceps* 8-10 hrs. after oviposition showing complete gastrulation ectoderm (ect), endoderm (end) and mesoderm (mes) at 400 X.

1.4. Organogenesis:

At 10 hours tracheae and spiracles were seen, at 11 hours the foregut was completely formed, in addition brain could be seen (Figure 6 a,b).

In addition, at 12 hours proventiculus and salivary glands were seen, Meanwhile, at 13 hours muscles were formed (Figure 6 c,d).

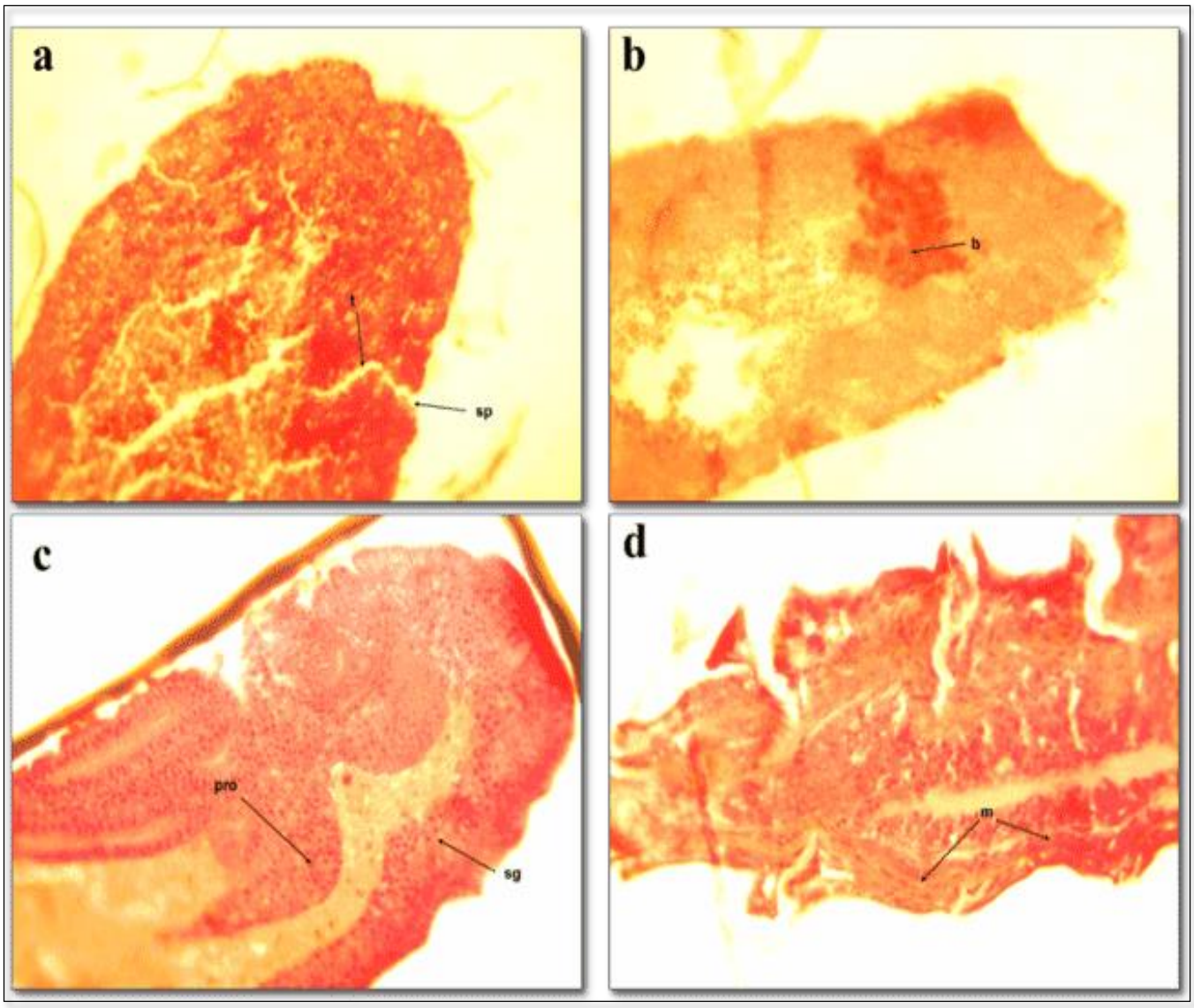

Figure 6. (a-d): (a) Sagittal section in untreated egg of *C. albiceps* 10 hrs. after oviposition showing tracheae (t) and spiracles (sp), 400 X, (b) Sagittal section in untreated egg of *C. albiceps* 11 hrs. after oviposition showing the brain (b) at 400 X, (c) Sagittal section in untreated egg of *C. albiceps* 12 hrs. after oviposition showing the alimentary canal, proventriculus (pro) and salivary glands (sg) at 100 X and (d) Sagittal section in untreated egg of *C. albiceps* 13 hrs. after oviposition showing formed muscles (m) at 400 X.

2. Histogenesis and differentiation as influenced by gamma radiation:

Sagittal sections from *C. albiceps* eggs laid by females emerged from pupae treated with sub-sterilizing doses of gamma radiation such as 4 and 10 Gy was investigated and compared with those of untreated ones (control).

Results revealed that the embryonic development of eggs treated with the sub-sterilizing doses (4 and 10 Gy) of gamma radiation was completed to gastrulation stage. A prolongation in the embryogenesis of treated eggs was recorded, where blastoderm was formed within 7 hours (Figure 7 a), compared to 5 hours for the untreated group. Gastrulation was attained within 12 hours from oviposition compared to 8-10 hours for control (Figure 7 b). Moreover, organogenesis continued normally, where the foregut and hindgut were formed within 13 hours compared with 10 hours in control eggs Also in this time tracheal system was seen (Figure 7 c,d).

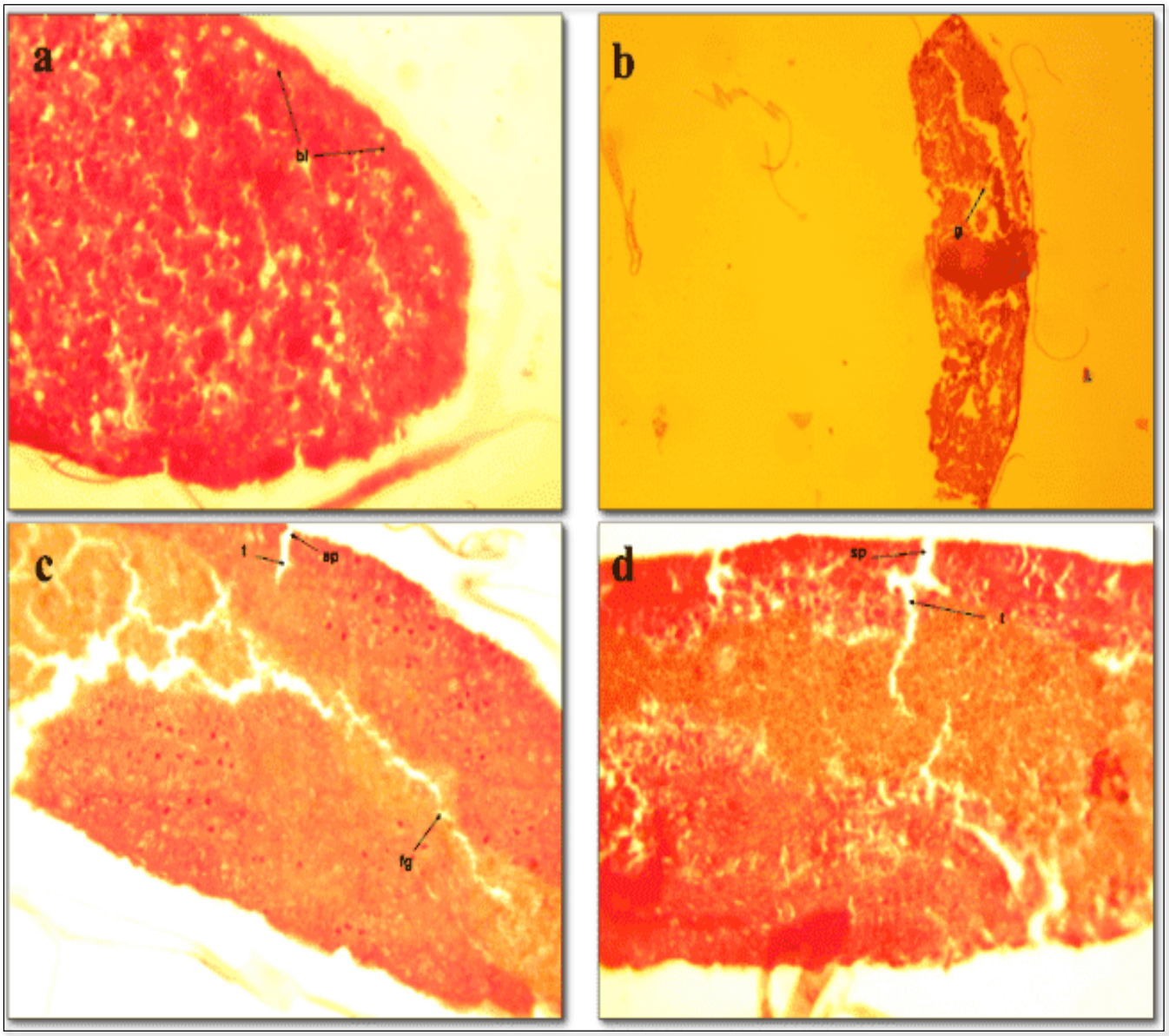

Figure 7. (a-d): (a) Sagittal section in irradiated egg (4 Gy) egg of *C. albiceps* 7 hrs. after oviposition showing blastoderm (bl) at 400 X, (b) Sagittal section in irradiated egg (4 Gy) egg of *C. albiceps* 12 hrs. after oviposition showing complete gastrulation (g) at 100 X, (c) Sagittal section in irradiated egg (4 Gy) egg of *C. albiceps* 13 hrs. after oviposition showing the fore gut (fg), trachea (t) and spiracles (sp) at 400 X and (d) Sagittal section in irradiated egg (4 Gy) egg of *C. albiceps* 13 hrs. after oviposition showing trachea (t) and spiracles (sp) at 400 X.

On the other hand, the higher doses of gamma radiation (10 Gy) the cleavage cells that migrate to the periphery of the egg to become merged within periplasm to form the blastoderm, were found to form an irregular cellular layer (Figure 8 a,b,c) after 8 hours from oviposition compared to 5 hrs. for untreated control. Moreover, the high dose (10 Gy) of gamma radiation was appeared to block the gastrulation (Figure 8 d).

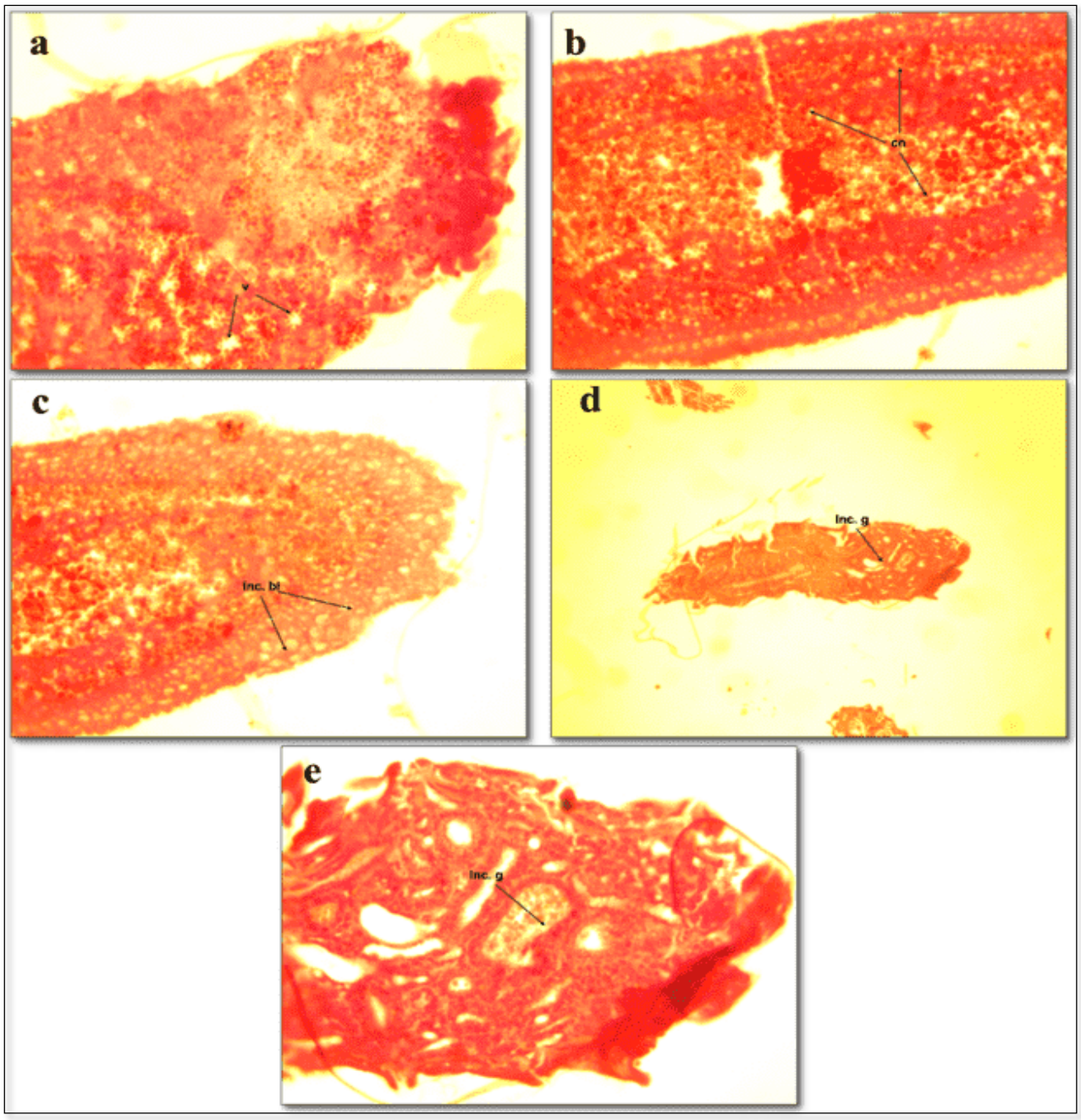

Figure 8. (a-e): (a) Sagittal section in irradiated egg (10 Gy) egg of *C. albiceps* 2 hrs. after oviposition showing vacuoles (v) at 400 X, (b) Sagittal section in irradiated egg (10 Gy) egg of *C. albiceps* 8 hrs. after oviposition showing the cleavage nuclei (cn) at 400 X, (c) Sagittal section in irradiated egg (10 Gy) egg of *C. albiceps* 8 hrs. after oviposition showing incomplete blastoderm (inc. bl) at 400 X, (d and e) Sagittal section in irradiated egg (10 Gy) egg of C. albiceps 12 hrs. after oviposition showing incomplete gastrulation (inc. g) at 100 X and 400 X, respectively.

Generally, there was significant variation ($P< 0.05$) between 10 Gy in comparison to control and 4 Gy group, while there was no significant differences ($P> 0.05$) between control and 4 Gy group (Figure 9 a,b).

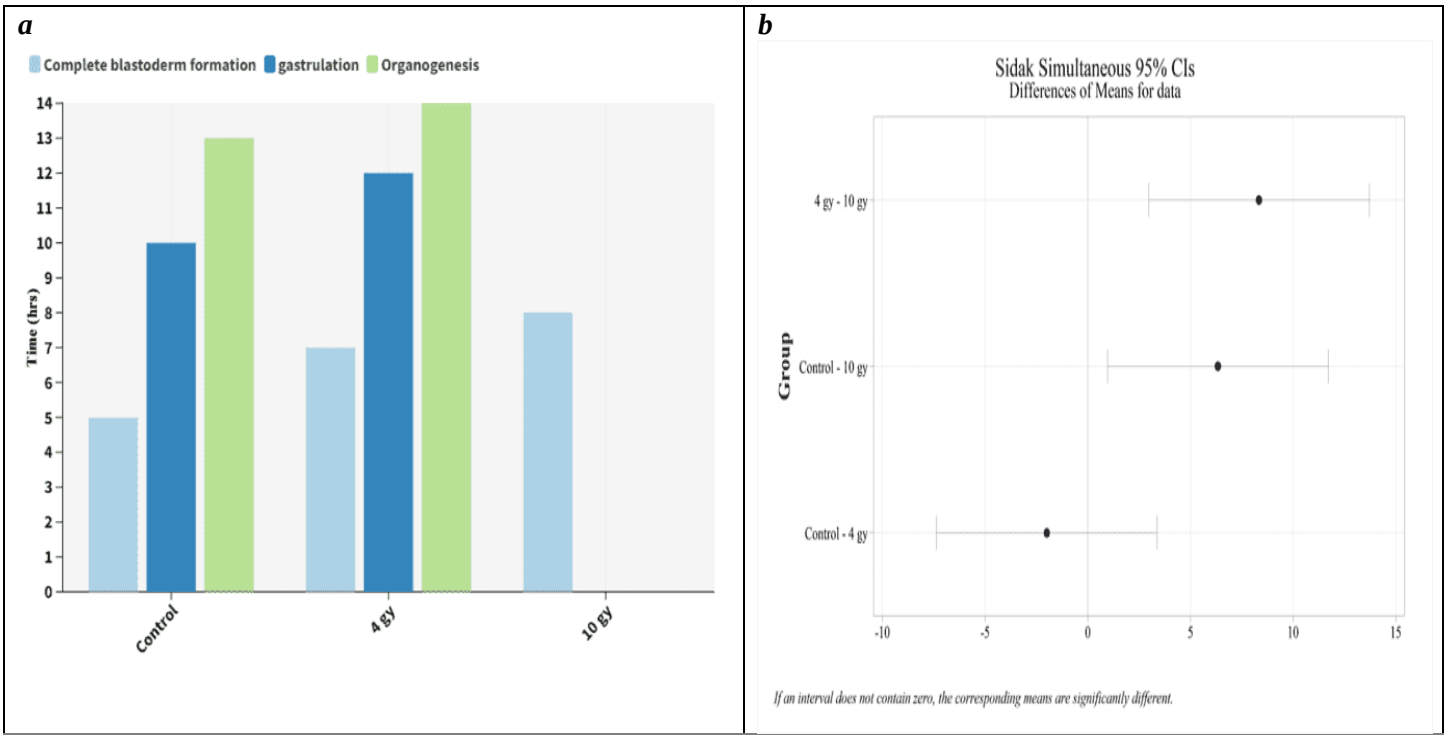

Figure 9. (a) Column chart represent the mean value for investigated groups, **(b)** Sidak simultaneous 95% CIs for differences of mean for investigated groups.

DISCUSSION

The present work was carried out to study the normal embryonic development of *C. albiceps* and anomalous development that may induced by treatment with gamma radiation. Events of the early stages of embryogenesis in *C. albiceps* may be summarized as the first cleavage nuclei were observed after 1 hour from oviposition, after about 2 and 3 hours, the cleavage nuclei became surrounded by a halo of cytoplasm and migrated towards the periphery of the egg. Also, after 4 hours, the cleavage cells were reached the periphery of egg leaving few nuclei in the center which form the vitellophages, almost all cleavage cells were merged within the periplasm to form continuous cellular layer or the "blastoderm" after five hrs. from oviposition. While, after 6 hours, the blastoderm was differentiated to form the germ band and the gastrulation was appeared as furrows in the germ band within 8 hours from oviposition. Finally, complete gastrulation was attained within 8-10 hours from oviposition.

In general, the embryogenesis of dipterous flies requires a shorter time for development as the time required for blastoderm formation in *Musca domestica* was 2.5 and 4.5 hours [(11)] at 25˚C, respectively compared with 1 and 5 days for blastoderm and gastrulation in *Eyprepocnemis plorans* at 28˚C, respectively.

Obtained results showed that, 4 Gy of gamma radiation induced a prolongation in the embryogenesis of *C. albiceps* as compared with control groups, where blastoderm was formed within 7 hours compared with 5 hours for the untreated group. Gastrulation was attained within 12 hours from oviposition compared with 8-10 hours for control. Moreover, organogenesis continued normally, where the foregut and hindgut were formed within 13 hours compared with 10 hours in control eggs. These results are in consistent with the previously reported results concerning with the effects of different chemical compounds on embryonic development of several dipteran flies, as benzamide (IGR) affect embryonic development of *M. domestica* and embryos of treated eggs continued in development until blastoderm stage [(12)], a great reduction in *Stomoxys calcitrans* and *Haemotobia irritans* treated with TH 6040 IGR (topical application) was recorded [(13)], UV-irradiation induced sterility in *Drosophila melanogaster* depending on the wavelength of UV light by frequency of pole-cell-deficient embryos, or as the frequency of agametic adults [(14)], hexaflumuron IGR affected *E. plorans* embryogenesis however low concentration didn't affect the sequence of embryogenesis [(15)] and radiation increase the number of *Drosophila* embryos containing apoptotic cells 75 min. after treatment with 3 Gy [(16)].

In addition, the higher dose of gamma radiation (10 Gy) inhibited the embryogenesis by blocking the gastrulation. Previously studies dealt with other stress factors agreed the obtained results of blocking gastrulation. Application of JHAs on insect eggs were found to inhibit the embryonic development in *Schistocerca gregaria* [(17)], *Dysdercus fasciactus* [(18)] and *Drosophila melanogaster* [(20)]. Also, hexaflumuron IGR stopped the sequence of embryogenesis of *Ey. plorans* at high concentrations [(15)].

CONCLUSION

Finally, we can conclude that low dose of gamma radiation (4 Gy) didn't affect the sequence and development of the embryo but caused a prolongation in the embryogenesis of treated eggs. Meanwhile, high dose of gamma radiation (10 Gy) inhibited the embryogenesis.

Conflict of Interests: The authors of this paper declare that they have no financial or personal relationships with individuals or organizations that would unacceptably bias the content of this paper and therefore declare that there is no conflict of interests.

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