

Protective Impact of Vitamin C Against Some Fetal and Neonatal Congenital Malformations and Anti-Inflammatory Non-Steroidal –Induced Hepatotoxicity of White Rats

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Abstract

The current study was designed to demonstrate the protective impact of vitamin C against some fetal and neonatal congenital malformations and anti-inflammatory non-steroidal–induced hepatotoxicity in them of white rats ,this study was carried out in animal house of biology department in Education Faculty for Girls -Kufa university from 1/11/2019 to 15/3/2020, 24 white female rats with age ranged between (11-12) weeks and weights ranged between (230-240) kg, in addition to (24) white male rats with ages between (9-11) weeks for mating, the female and male rats were placed together for mating, after obtaining the required number of pregnant female rats, these pregnant rats were distributed to 4 groups , each group included 6 pregnant rats ,the group1 was treated with normal saline solution and was the control group, group2 was treated with indomethacin drug with a concentration of 8.40 mg / kg of body weight, while group 3,4 were treated with vitamin C with a concentration of 85 mg /kg of body weight and vitaminC with a concentration of 85 mg / kg of body weight + indomethacin drug with a concentration of 8.40 mg / kg of body weight respectively, pregnant female rats in all groups were treated orally from the first day of pregnancy, 3 pregnant rats were dissected during 20 days of pregnancy, while the remaining 3 pregnant rats were left to after birth of each group.

The results of the this study pointed to that a significant decrease ($P < 0.05$) in numbers of total and living of the embryos for pregnancy period (20) days and newborns

Keywords: -Vitamin C, ascorbic acid, indomethacin, hepatotoxicity, female rats, fetal malformations.

after birth, while a significant increase ($P < 0.05$) in numbers of dead of the embryos for pregnancy period (20) days and newborns after birth, and the results showed pathohistological changes in livers of of the embryos for pregnancy period (20) days and newborns after birth in the group treated with indomethacin compared with control group and groups treated with vitamin C and vitamin C + indomethacin respectively , while the results demonstrated that a significant increase ($P < 0.05$) in numbers of total and living while a significant decrease ($P < 0.05$) in numbers of dead of the embryos for pregnancy period (20) days and newborns after birth in groups treated with vitamin C and vitamin C + indomethacin respectively comparing with the control group, while the study accounts did not show any significant differences ($P > 0.05$) in numbers of total, living and dead of the embryos for pregnancy period (20) days and newborns after birth in these two groups when compared between them , and there were no pathohistological changes in livers of the embryos for pregnancy period (20) days and newborns after birth in groups treated with vitamin C and vitamin C + indomethacin comparing with the control group respectively.

Introduction

Vitamins are one of the main antioxidants that have the ability to reduce or prevent negative effects with regard to fetal malformations and birth defects, the most important of these vitamins is vitamin C or so-called ascorbic acid which it is one of the important vitamins and available in plasma at high levels providing great protection of the humans and animals (Marriott *et al.*, 2020), vitamin C is a water-soluble vitamin that was discovered in 1912 and isolated in 1928 (Squires, 2011), and it performs basic and vital functions in the body such as building bone and tissue bonding and blood vessels, collagen formation, healing of wounds, strengthening the immune system, helps in the generation of some other antioxidants such as vitamin E, synthesis of some enzymes as a co-factor, reducing the effectiveness of these enzymes in digesting important body molecules such as DNA, proteins, fats and others (Bjelakovic *et al.*, 2012), in addition to its role as an effective and important antioxidant in reducing oxidation reactions or preventing them by inhibition the reaction chains that stimulate the process of oxidation in cells leading to remove the oxidative stress by its work as an attractant agent of free oxygen radicals and transforming them into ineffective compounds and thus preventing their various harmful effects, many studies indicated that the drugs generate oxidative stress that arises from their ability to form and produce free radicals that cause the destruction of the body's various cells and organs (Maqbool *et al.*, 2018).

Indomethacin is a drug that belongs to the anti-inflammatory drugs (NASIDs) which were used to treat inflammations, reduce fever and relieve pain by its action to inhibit cyclooxygenase enzymes which stimulate the production of prostaglandins which are essential mediators in fever, pain, and the inflammation in the body (Modi *et al.*, 2012), since the discovery of indomethacin drug had treated many diseases such as various joint infections , gout, headaches, renal colic , diabetes insipidus and Pagitt's disease (Crofford, 2013), and this drug reduces the fetal amniotic fluid when it is inflamed, causes delayed delivery and lengthens pregnancy by stopping early labor, as well as for the treatment of menstrual disorders in females (Namieta *et al.*, 2000), some studies have shown that prostaglandins produced by COX enzymes perform effective functions in various body organs such as protection of the mucous layer of the gastro-intestinal tract, maintaining renal function and preventing thrombocytopenia causing prolonged bleeding time and others (Modi *et al.*, 2012), therefore the inhibition of these enzymes synthesis by indomethacin and other NASIDs stimulates many negative effects in the body that increase in intensity with increasing drug concentration and duration of administration, especially in pregnant women and fetuses during pregnancy, mothers and newborns after birth, so antioxidants in general and foodstuffs in them in particular play major actions in reducing or preventing toxicity. Pharmacokinetics by protecting them from the harmful effects of the oxygen free radicals generated by

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these drugs which negatively affect mothers, fetuses and newborns (Phaniendra *et al.*, 2015) and for the purpose of highlighting on the efficiency of vitamin C in the protection against some fetal and embryonic congenital malformations and hepatic toxicity stimulated by indomethacin in the livers of fetuses during pregnancy and newborns after birth of female rats, this study was planned.

Materials and Methods

Laboratory animals

The study was performed in the animal house of Education College for Girls - university of Kufa, (24) white female rats *Rattus rattus* (Sprague dawley) with age ranged between (11-12) weeks and weights ranged between (230-240) kg, in addition to (24) white male rats of the same type with ages between (9-11) weeks, all females and males were placed in special plastic cages and under the same laboratory conditions of humidity, ventilation and temperature (20-25) C° and lighting for a period 14 hours, animal rats were provided freely with water and food during each study period.

Vitamin C

Special vitamin C of laboratory animals was brought from the laboratory equipment offices, then the required concentration in this study was attended (85 mg / kg of body weight).

Indomethacin drug

The drug was brought from pharmacies in form of capsules, each one capsule contained (25 mg), then the required concentration in the current study was 8.40 mg / kg of body weight prepared.

Mating of animals

In mating cages, female rats were placed after the estrus unification in them with the male rats, and with reality one male for each female in the evening and in the morning the female rats were examined to see the mating plug in the female vagina or on the floor of the cages, and then vaginal swabs were also made to observe the sperms after staining them with blue methylene to make sure of mating female rats, and on the day when the mating plug or sperm appears in the female vagina, it is the zero day of pregnancy (Yaping *et al.*, 2006).

Animal division

24 pregnant female rats were divided into 4 large groups, each group was contained 6 pregnant rats, the first group was administered with a physiological salt solution (control group), and the second group was administered with indomethacin at a concentration of 8.40 mg / kg of body weight, while the third group was treated with vitamin c at a concentration of 85 mg / Kg of body weight and the fourth group was administered with vitamin C at a concentration of 85 mg / kg of body weight and after 30 minutes passed it was administered with indomethacin at a concentration of 8.40 mg / kg of body weight, then each large group was divided into two subgroups, each of them included 3 pregnant rats, after 20 days of pregnancy have passed, 3 pregnant rats were dissected for the fetal study, while the remaining 3 pregnant rats were left to after birth for the neonatal study of each group, all pregnant female rats were administered daily one dose from the first day of pregnancy by oral stomach tube of rats.

Animals anatomy

Female rats weights were recorded before pregnancy (before treatment) and after birth (after treatment), body weights were measured during the 20 day of the pregnancy using a weight balance, after that pregnant

rats were drugged in a 20 day of pregnancy by diethylether, then the abdominal cavity of pregnant rats was opened, ovaries and uterine horns (right and left) were obtained of different treatments, the numbers of total,alivingand dead of embryos and their numbers in each uterine horn were counted,then the uterine horns were opened by sharp scissors and the embryos were washed with distal water and dried by filter papers, and as well as after obtaining the newborns after birth, the numbers of total,alivingand dead of newborns were recorded, after that the body weights of embryos and newborns were calculated by the weight balance, then the embryos for 20 days of pregnancy and newborns after birth were anesthetized by sufficient quantity of diethylether and vivisected by opening the abdominal cavity of them and the livers were removed and weighed by the sensitive electrical balance, then the livers werer placed in the formalin solution at a concentration of 10% for a period 48 hours for fixing them and preparing the histological sections of the livers.

Preparation of liver tissue sections of embryos and newborns

The histological sections of liver of the embryos for a pregnancy period 20 days and newborns after birth were prepared according to the method of Suvarna *et al* (2013), these sections were examined by compound microscope - Japan and then photographed with the same examination microscope after being supplied it with a camera containing a color film.

Statistical analysis

Data of present study were analyzed using (SPSS),the values were as Mean \pm Standered Error (M \pm SE) which were elicited by (F-Test),and was used least significant diffence (L.S.D) at significant level for finding significant differences between the study groups.

Results and discussions

Effect of study groups on some fetal and neonatal parameters

Table (1) showed that the rats treated with indomethacin suffered from a significant decrease (P <0.05) in the numbers of total and living embryos and newborns after birth, but the numbers of dead embryos and newborns were significantly increased (P <0.05) when compared with untreated rats and other treatment groups respectively and as shown in the figure (1), while the results in the same table(1) recorded a significant increase (p <0.05) in the numbers of total and living embryos and newborns after birth, while there were a significant decreased (P <0.05) in numbers of dead embryos and newborns in groups of animals that treated with vitamin C and vitamin C + indomethacin respectively when compared with the control group, while there no significant differences (P > 0.05) between these two groups in above parameters, and no significant differences (P > 0.05) in the numbers of living and dead embryos and newborns when comparing these two groups with control group or when compared between them as shown in the figures (2,3,4) respectively, these results of present study may be back to that indomethacin drug has an effect in inhibition of the effectiveness of cyclooxygenase enzymes (COX-1, COX2) which were responsible for the synthesis of prostaglandins that play effective and essential

roles in various sexual activities such as ovulation, pregnancy and implantation, as they play important

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functions in the vascular permeability of the inner lining of the uterus in the process of implantation and duciation, therefore, the inhibition of these enzymes cause negative effects on the processes of ovulation, implantation, growth and fetal development (Damaceno *et al.*, 2008), or these results may be due to the increase in implant loss of pregnant rats when the indomethacin was administered which reduced the numbers of living embryos while increased the numbers of absorbed and dead embryos and the numbers of stillbirths after birth because this drug stimulated serious complications of embryos and newborns after birth such as high pulmonary pressure and patent arterial ducts and intracranial hemorrhage and urinary disorders, in addition to necrotizin enterocolitis (Eronen *et al.*, 1994), or the cause of these results could be due to the oxidative stress resulting from the generation of free oxygen radicals, especially the reactive species of oxygen (ROS) by the indomethacin drug which increases oxidative breakdown as a result of oxidation of fats in cellular membranes and vitally important molecules such as DNA, proteins, etc. in fetal tissues causing the failure of implantation and the evolutionary processes of embryos and newborns after birth later (Iborre *et al.*, 2005), while the reasons of the significant increase in the numbers of total and living embryos and newborns and the significant decrease in the numbers of dead embryos and newborns in animals treated with vitamin C and vitamin

C + indomethacin were that vitamin C is an important antioxidation (wang and Xu, 2016) providing the protection for the horns of the uterus and the existing embryos in them from the oxidative stress that arose from the reactive oxygen species (ROS) which stimulated the breakdown in the tissues of the embryos of rats treated with indomethacin causing bad effects in the fetal development during the different stages of pregnancy, especially in the 20 day period of pregnancy, so the administration of vitamin C of pregnant rats from the beginning of pregnancy led to an increase in implantation of embryos and the number of developed and living embryos and their protection from oxidative stress because this vitamin interacts with these free radicals and removes them before they reach to the embryos causing increased implantation and living numbers of embryos and then the newborns and thereby diminishing the loss of implantation and numbers of embryos adsorbed and then the stillbirth, or this result may be explained by the role of vitamin C in reducing or preventing (Yildiz *et al.*, 2016) the effects of indomethacin which resulted from the inhibition the production of prostaglandins that contribute to the perpetuation of the loaded blood flow with necessary nutrients for various reproductive processes such as implantation and fetal development during the various stages of pregnancy which was reflected positively on newborns after the birth.

Table 1. Effect of study groups on some fetal and neonatal parameters.

Parameters\Group s	The numbers of					
	Total		Living		Dead	
	Embryos	Newborns	Embryos	Newborns	Embryos	Newborns
G1	8.75 ± 0.02	8.78 ± 0.05	8.75 ± 0.03	8.78 ± 0.01	0.00 ± 0.0	0.00 ± 0.0
G2	4.00 ± 0.01 ab	3.12 ± 0.05 ab	2.11 ± 0.01 ab	1.07 ± 0.06 ab	2.52 ± 0.04 ab	1.20 ± 0.08 ab
G3	9.88 ± 0.01 a	9.96 ± 0.03 a	9.88 ± 0.04 a	9.96 ± 0.07 a	0.00 ± 0.00a	0.00 ± 0.00
G4	9.73 ± 0.09 a	9.86 ± 0.02 a	9.73 ± 0.01 a	9.86 ± 0.01 a	0.00 ± 0.00a	0.00 ± 0.00
L.S.D	0.18	0.00	0.19	0.09	0.08	0.06

Values: Mean ± Standard Error

G1: Control group.

G2: group treated with indomethacin at a concentration of 8.40 mg / kg.

G3: Group groups treated with vitamin C at a concentration of 85 mg / kg.

G4: groups treated with vitamin C at a concentration of 85mg/kg

+ indomethacin at a concentration of 8.40 mg / kg.

a: represents a significant difference for the control group with a significant level (P < 0.05).

b: represents a significant difference from other groups with a significant level (P < 0.05).

L.S.D: Least Significant Difference with significant level (P < 0.05).

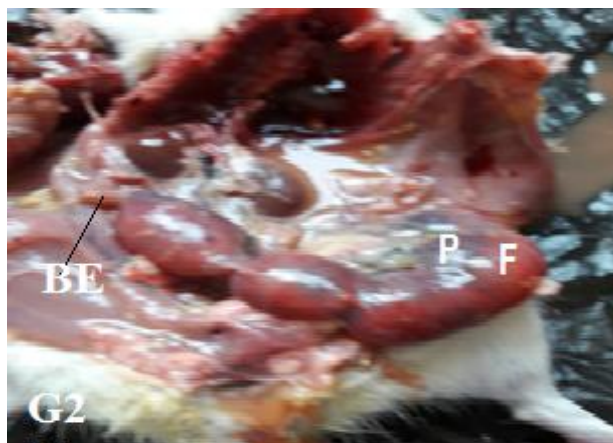


Figure 1. Dissected pregnant female rats of control group (G1) for 20 days of pregnancy showing that: the embryos in the utero. Notice embryo (F) in the utero, the placenta (P).

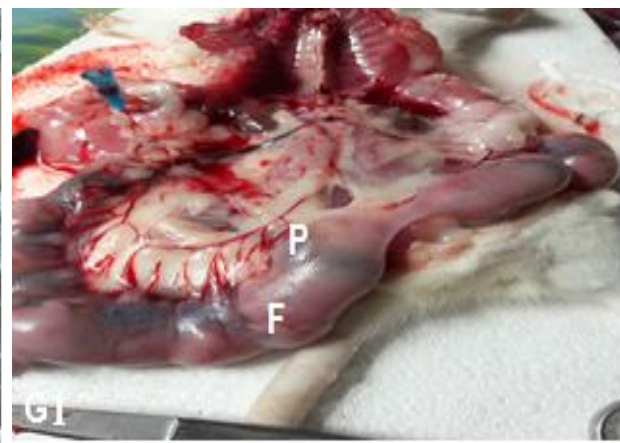


Figure 2. Dissected pregnant female rats of treated group with indomethacin (G2) for 20 days of pregnancy showing that: the small embryos in the utero (F) in the utero, the small placenta (P), absorbed embryo (BE).

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Effect study groups on the weights of the body and livers and on hepatic-histological changes of the embryos for a pregnancy period of 20 days and newborns after birth.

The results of our current study pointed to a significant decrease ($P < 0.05$) in weights of body and liver of embryos for a pregnancy period of 20 days and newborns after birth in group that were administered with indomethacin (Table 2), as patho-histological changes were observed such

as separation the hepatic capsule about hepatic –tissue , loss the formation and regular arrangement of hepatic-tissue, vascular dilation and congestion, haemolysis, infiltration of inflammatory cells, necrosis and finally degeneration in hepatic- tissue of embryos, while increased the intensity of these histological changes in the livers of newborns after the birth and as in figures (6,7,8,9,10,14,15,16,17,20) respectively when compared with control group and with the other administered groups , the cause of these results may be due to the created prostaglandins by activity of cox1 and



Figure 3. Dissected pregnant female rats of group with vitamin C (G3) For 20 days of pregnancy showing that: the embryos in the uterus. Notice embryo (F) in the uterus, the placenta (P).

cox2 enzymes which have important effects on the functions of the reproductive system in addition to their participation in vascular functions of the blood vessels as vasodilating factors that allow the delivery of nutrients and oxygen to various tissues of the body, therefore the inhibition of the biosynthesis of prostaglandins by indomethacin leads to vasoconstrictive effects causing impaired blood flow during the placenta-uterine circulation and consequently less transport of nutrients to embryos stimulating different deformities in body organs such as the kidneys (AL-Essawi and Aljamali, 2019a) and promoting lack of body weights of embryos and which reflected on the weights of newborns after birth, or the reason for this result could be attributed to the fact that indomethacin drug raised the oxidative stress due to the increase in the formation of free radicals of oxygen that led to the oxidative breakdown of all body organs simulating the harmful effects in the different pregnancy outputs especially the growth and development of embryos causing lack of their body weights which later affected the newborns weights, or since the loss of balance between the products of

oxidation and anti-oxidants that were poorly developed in the early stages of pregnancy leads to early pregnancy failure and delayed fetal development during the advanced stages of pregnancy causing distorted effects and cytotoxicity in the embryos of pregnant rats by preventing the development and growth of different body systems, especially in skeletal elements causing low body weights (Al-essawi and Aljamali, 2019b), and the significant decrease in weights of livers and structural changes in the tissue in them of embryos and newborns and because there were no studies to explain the results of our research, therefore these results can be explained by the ability of cyclooxygenase enzymes which produce prostaglandins in the embryo and newborn livers which may play principal roles in the development and growth of livers during the different stages of pregnancy and maturity changes in the livers of newborns, therefore the changes that were observed in the histological structure in livers of the embryos and newborns of these groups may be due to the effectiveness of indomethacin in generating the various free radicals of oxygen (May *et*



Figure 4. Dissected pregnant female rats of group with vitamin C + indomethacin (G4) for 20 days of pregnancy showing that: the embryos. Notice embryos (F) in the uterus, the placenta (P).

al., 2003) which stimulate the oxidative breakdown and destruction of the fats in cellular membranes and others large molecules such as DNA and proteins leading to destruction and damage of advanced tissues in the livers of embryos during pregnancy, especially in the last stage of it and newborns and thus loss of their weights, or the results of this study may be explained to the activity of indomethacin on inhibition cox enzymes which produce the prostaglandins that represent vasodilating agents and supply the blood flow of different organs like livers carrying with it the main nutrients of them, therefore, the failure of production these compounds would promote vasoconstriction effects and ischemia causing loss or low of blood supply in them which stimulates pathological changes in the histological component of the livers that have been noticed in embryos and increased severity in the newborns after birth whose their mothers were treated with indomethacin which has a toxic effect during fetal life and after birth.

On the contrary, animal groups that treated with vitamin C and vitamin C + indomethacin showed a significant increase ($P < 0.05$) in weights of body and livers (Table

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2) ,as the histological study did not point to abnormal changes of liver tissues in embryos for a period 20 days of pregnancy and newborns after birth as in figres (11,12,20,22)respectively compared with control animal rats (5,13)respectively , and these results may be back to that vitamin C is a powerful and effective antioxidant(Ghate *et al* .,2011)and represents a first line of defense in the body against the free radicals ,especially the reactiv oxygen species(ROS)that produce from the oxidation of fats and others molecules in various body organs such as the livers causing oxidative breakdown in embryos and newborns later,so vitamin C had protected body organs from programmed death which was stimulated by indomethacin in different body tissues like the hepatic cells by neutralizing these free radicals and rid the cells of them and prevent their arrival to embryos and newborn organs ,thus vitamin C preserved the histological intergrity of them and prevented or reduced the toxicity of indomethacin and caused the elevation in weights of body and livers of embyros and newborns after the birth, or this result can be explained on the basis

that vitamin C strengthened the vascular function of the blood vessels in the liver and provided protection for the hepatic tissue from low blood perfusion (Yildiz *et al* .,2016) which was generated it indomethacin drug by the inhibition the production of prostaglandins that act as vasodilator agents of the blood vessels, thus vitamin C promoted the blood flow to the livers and brought with it the necessary nutrients of their various functions, therefore vitamin C promotes the biosynthesis of prostaglandins which maintain hepatic blood flow and stimulating the growth and develoment of the hepatic tissue in embyros and newborns. Consequently, vitamin C is a protective factor against the actions of low blood perfusion and oxidative stress that resulted from poisoning with indomethacin drug for its role in the production of prostaglandins and also rid the body of free oxygen radicals, thus repeating the balance between oxidizive agents and antioxidant factors and stimulating the development and formation of hepatic tissues in embyros and newborns and increases their weights.

Table 2. Effect of study groups on the weights of livers of embyros for a pregnancy period 20 days and newborns after birth.

Parameter\Grou ps	Embyros			Newborns		
	body weight(g m)	Liver weight (gm)	liver weight / body weight	body weight(gm)	Liver weight (gm)	liver weight / body weight
G1	5.41±0.02	0.15±0.03	0.16±0.03/5.41±0.02	5.69±0.04	0.16±0.05	0.16±0.05/5.69±0.04
G2	3.00±0.03	0.11±0.01 ab	0.11±0.01/3.00±0.03	2.42±0.04	0.10±0.02ab	0.10±0.02/2.42±0.04
G3	5.83±0.02	0.18±0.06 a	0.18±0.06/5.83±0.02	5.85±0.01	0.19±0.041 a	0.19±0.041/5.85±0.01
G4	5.80±0.05	0.17±1.02 a	0.17±0.04/5.80±0.05	5.83±0.05	0.17±0.01 a	0.17±0.01/5.83±0.05
L.S.D	0.21	0.02		0.10	0.01	

Values: Mean ± Standard Error.

G1: Control group.

G2: The group treated with indomethacin at a concentration of 8.40 mg / kg.

G3: Group group treated with vitamin C at a concentration of 85 mg / kg.

G4: group treated with vitamin C at a concentration of 85 mg / kg

+ indomethacin at a concentration of 8.40 mg / kg.

a: represents a significant difference for the control group with a significant level (P <0.05).

b: represents a significant difference from other groups with a significant level (P <0.05).

L.S.D: Lowest Significant Difference with significant level (P <0.05).

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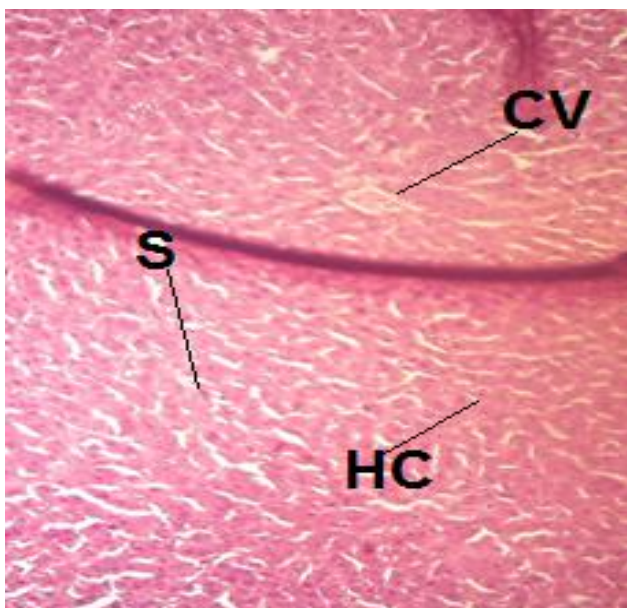


Figure 5. Section of rat embryo liver tissue for pregnancy period (20) days of control group showing that s:- normal histomorphology of general architecture from:-Central Vein (CV),Sinusoids(S),Hepatic Cell (HC) (H& E 100 X)

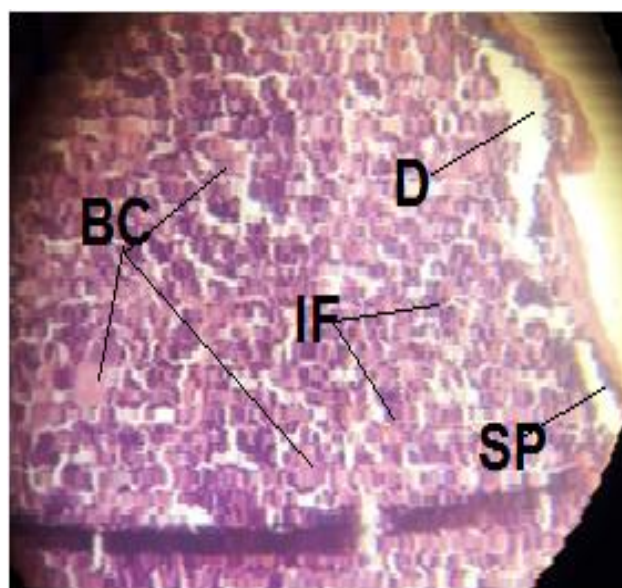


Figure 6. Section of rat embryo liver tissue for pregnancy period (20) days of treated group with indomethacin showing that:-degeneration hepatic tissue (D), separation hepatic capsule (SP),infiltration of inflammatory cells (IF),blood agglutination(CB).(H& E 100 X)

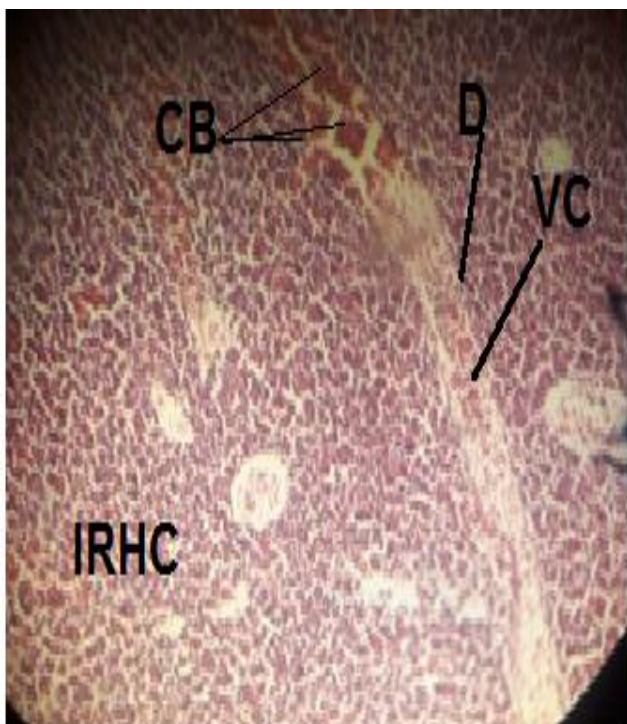


Figure 7. Section of rat embryo liver tissue for pregnancy period (20) days of treated group with indomethacin showing that: irregular arrangement of hepatocytes (IRHC), dilation (D) and vascular congestion (VC), blood agglutination(CB) (.H& E 100 X).

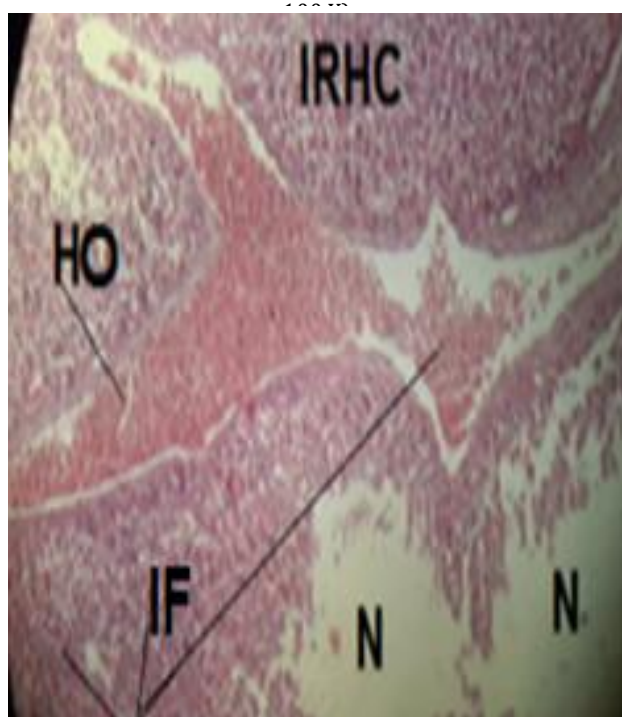


Figure 8. Section of rat embryo liver tissue for pregnancy period (20) days of treated group with indomethacin showing that:- irregular arrangement of hepatocytes (IRHC),necrosis in hepatic tissue (N),infiltration of inflammatory cells (IF),hemorrhage in hepatic tissue(HO).(H& E 100 X).

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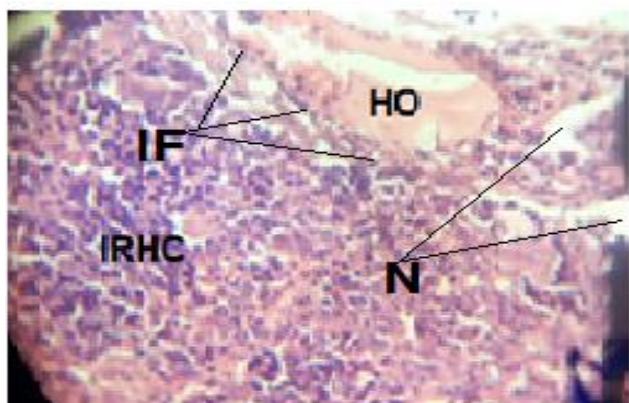


Figure 9. Section of rat embryo liver tissue for pregnancy period (20) days of treated group with indomethacin showing that:- irregular arrangement of hepatocytes (IRHC), necrosis in hepatic tissue (N), infiltration of inflammatory cells (IF), vascular dilation and haemolysis (H). (H& E 100 X).

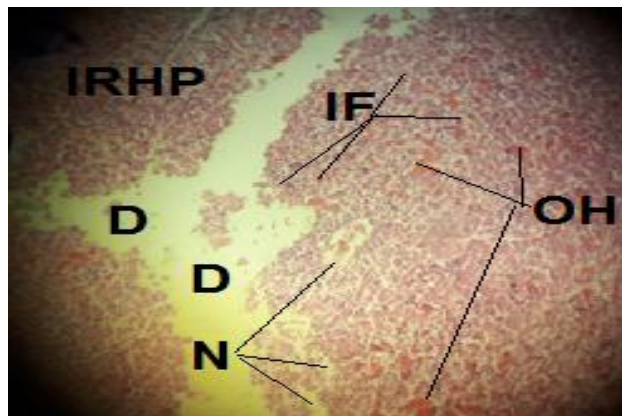


Figure 10. Section of rat embryo liver tissue for pregnancy period (20) days of treated group with indomethacin showing that:- irregular arrangement of hepatocytes (IRHC), increased necrosis in hepatic tissue (N), increased infiltration of inflammatory cells (IF), degeneration of hepatic tissue (D). (H& E 100 X).

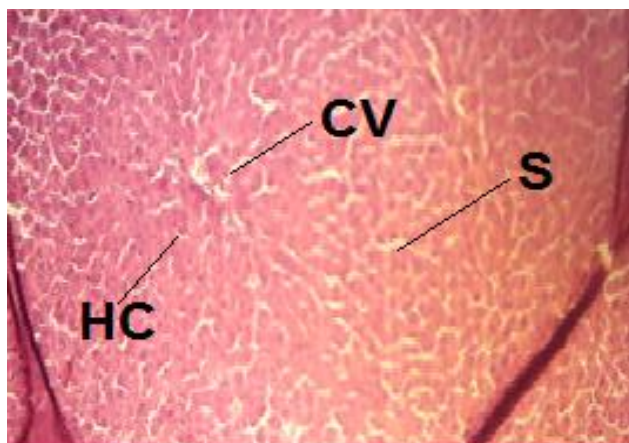


Figure 11. Section of rat embryo liver tissue for pregnancy period (20) days of treated group with vitamin C showing that:- normal histomorphology of general architecture from:-Central Vein (CV), Sinusoids (S), Hepatic Cell (HC) (H& E 100 X).

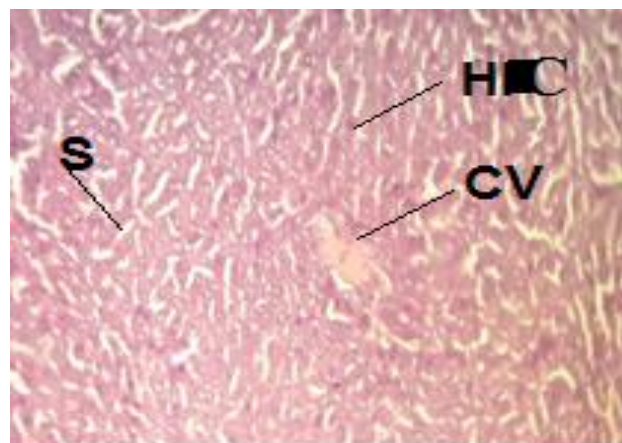


Figure 12. Section of rat embryo liver tissue for pregnancy period (20) days of treated group with vitamin C + indomethacin showing that:- normal histomorphology of general architecture from:-Central Vein (CVP), Sinusoids (S), Hepatic Cell (HC)).(H& E 100 X).



Figure 13. Section of rat newborn after birth liver tissue of control group showing that: - normal histomorphology of general architecture from: -Central Vein (CVP), Sinusoids(S), Hepatic Cell (HC). (H& E 100 X).

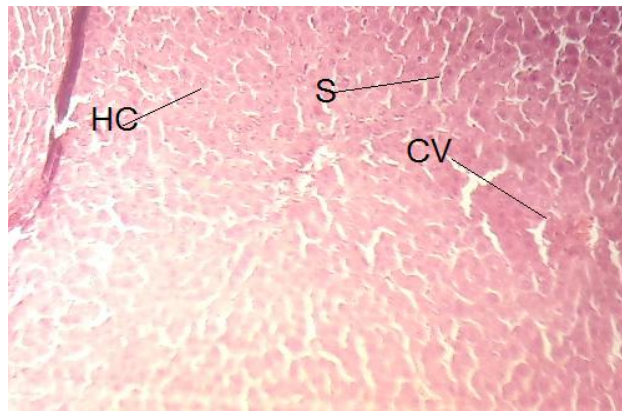


Figure 14. Section of newborn after birth liver tissue of treated group with indomethacin showing that:- increasing degeneration of hepatic tissue (D), increasing separation of hepatic capsule (SP), necrosis in hepatic tissue (N), expansion of sinusoids (ES). (H& E 100 X).

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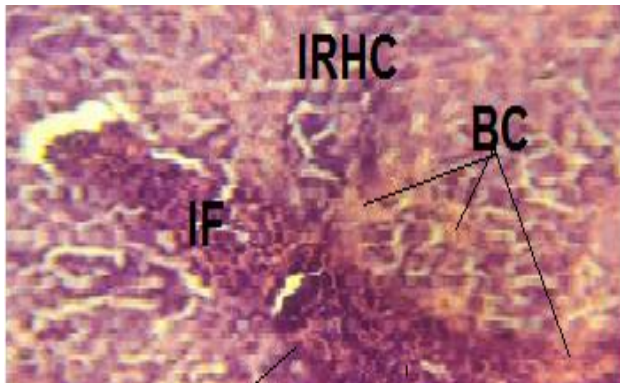


Figure 15. Section of rat newborn liver tissue of treated group with indomethacin showing that:-irregular arrangement of hepatocytes(IRHC),increasing infiltration of inflammatory cells (IF),increasing vascular congestion (VC),blood agglutination(BC).(H& E 100 X).

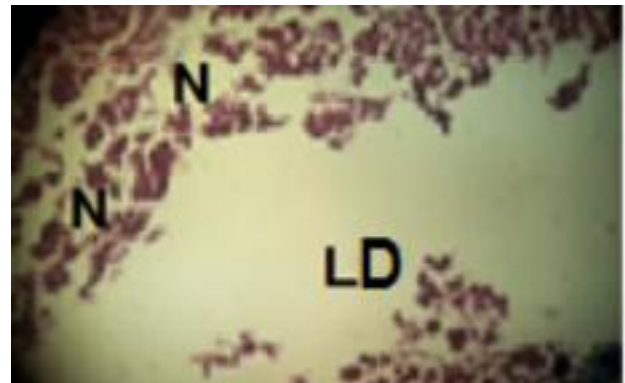


Figure 16. Section of rat newborn liver of treated group with indomethacin showing that: - irregular arrangement of hepatocytes (IRHC), increased necrosis in hepatic tissue (N), loss and degeneration of hepatic tissue (LD). (H& E 100 X).

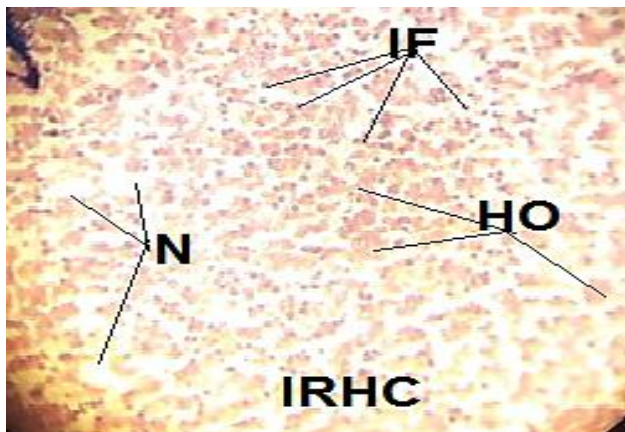


Figure 17. Section of rat newborn liver tissue of treated group with indomethacin showing that:- vacuolation in hepatocytes(V),necrosis in hepatic tissue (N),infiltration of inflammatory cells (IF),increasing vascular congestion (VC),expansion of sinusoids(ES).(H& E 100 X).

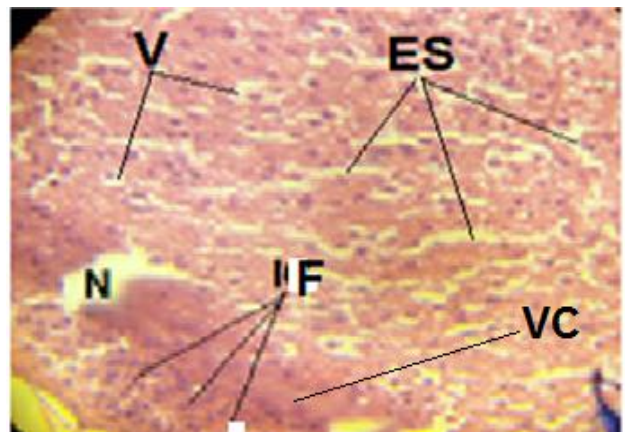


Figure 18. Section of rat newborn liver tissue of treated group with indomethacin showing that:- irregular arrangement of hepatocytes(IRHC), necrosis in hepatic tissue(N), infiltration of inflammatory cells (IF),increasing hemorrhage in hepatic tissue (HO) .(H& E 100 X).

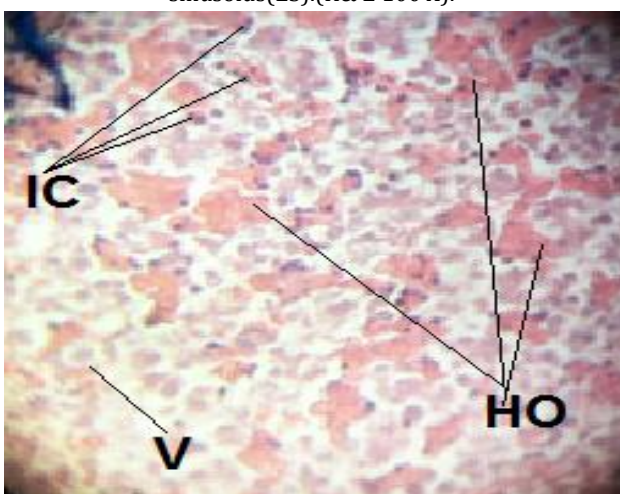


Figure 19. Section of rat newborn liver tissue for of treated group with indomethacin showing that: - increasinginfiltration of inflammatory cells (IF), vacuolation in hepatocyte(V), severe hemorrhage in hepatic tissue (HO). (H& E 100 X).

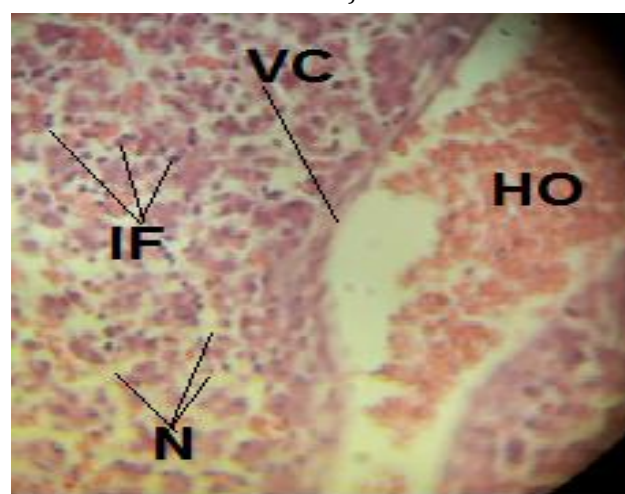


Figure 20. Section of newborn after birth of treated group with indomethacin showing that: - necrosis in hepatic tissue (N), infiltration of inflammatory cells (IF), vascular congestion (VC) with hemorrhage (HO) .(H& E 100 X).

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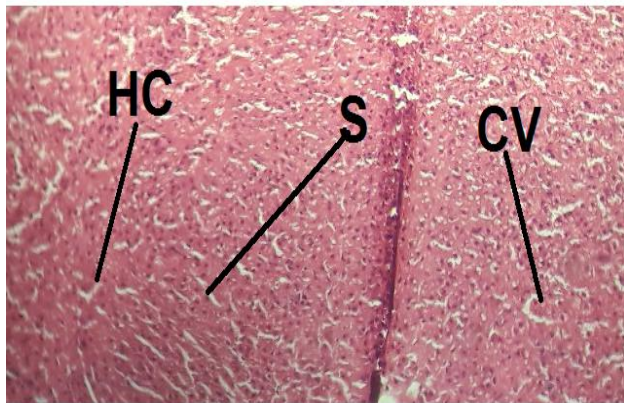


Figure 21. Section of rat newborn after birth liver tissue of treated group with vitamin C showing that :- normal histomorphology of general architecture from:-Central Vein (CVP), Sinusoids(S), Hepatic Cell(HC)).(H& E 100 X).

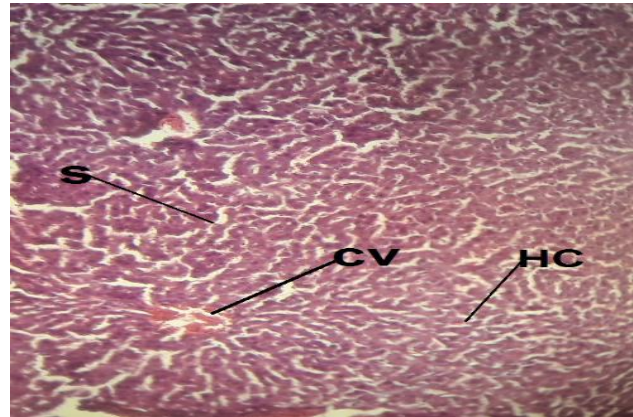


Figure 22. Section of rat newborn after birth liver tissue of treated group with vitamin C +indomethacin showing that: - normal histomorphology of general architecture from: -Central Vein (CVP), Sinusoids (S), Hepatic Cell (HC). (H& E 100 X).

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