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Safety and Efficacy of a Developed 'Patent Ductus Arteriosus Occluder Device': A Porcine Model Analysis Over Multiple Time Points

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Abstract:- This study assessed the safety and performance of a patent ductus arteriosus (PDA) occluder using a porcine carotid artery model involving three male pigs. Each pig underwent a thorough physical examination and met the criteria for stable health and body weight. On day 0, the occluder was implanted under proper analgesia and anesthesia, with heparin used to prevent clot formation. The deployment process was meticulously evaluated through angiography and Quantitative Vascular Analysis (QVA) before and after implantation. Arteries were harvested at 90, 180, and 365 days for histopathological analysis. The occluder fulfilled all acceptance criteria, demonstrating successful deployment, trackability, and no cranial artery flow above the device. Gross necropsy showed a bulged carotid artery, suggesting a reaction to the device, while histopathological evaluations showed good endothelialization at all time points. The occluder was well-visualized during imaging, deployed easily, and could be withdrawn without issue. It effectively occluded the artery at all follow-ups without migrating from the target site. All animals survived the procedure and post-procedural period with the device intact. These results suggest that the PDA occluder is safe and effective in this model, showing promise for future human clinical applications, though further studies are needed to confirm long-term safety and efficacy.

Keywords:- Patent Ductus Arteriosus (PDA) Occluder Device, Porcine Model, Safety Evaluation, Performance Assessment, Radiography And Angiographic Findings, Histopathological Findings.

I. INTRODUCTION

In this study, we aimed to assess the safety and performance of the developed Patent ductus arteriosus (PDA) is a congenital heart defect characterized by the persistence of the ductus arteriosus after birth, resulting in abnormal blood flow between the aorta and the pulmonary artery. Left untreated, this condition can lead to significant complications, including heart failure and pulmonary hypertension. Consequently, various occlusion devices have been developed to close the PDA, aiming to prevent these complications and improve patient outcomes.

This study evaluates the safety and performance of a novel device, the patent ductus arteriosus Occluder, designed to achieve effective closure of the PDA. The investigation adhered to ISO standards for circulatory support devices, ensuring rigorous evaluation protocols.

Three male pigs were selected for this study, which involved a series of procedures and assessments over a one-year period. The animals were fasted overnight before each procedure to ensure optimal conditions for anesthesia and surgery. Pre-study physical examinations verified the health and suitability of the pigs, with no significant clinical observations or weight loss recorded.

On day 0, under appropriate analgesia and anesthesia, the PDA Occluder was implanted into the carotid artery. Heparinization was administered to prevent clot formation. Pre- and post-implantation assessments included angiography and quantitative vascular analysis (QVA) to evaluate the device's deployment and immediate effectiveness. The study design incorporated sequential sacrifices and arterial harvests at 90, 180, and 365 days post-implantation for comprehensive histopathological evaluation.

Primary endpoints for the study included proper deployment of the occluder, animal survival, and device performance over time. Secondary endpoints focused on the visualization and trackability of the device post-deployment, cessation of blood flow at the cranial artery site, and endothelialization of the occluded artery.

By conducting this thorough evaluation, the study aim to provide substantial evidence on the safety and efficacy of the Patent ductus arteriosus Occluder, potentially offering a reliable solution for PDA closure and improving clinical outcomes for patients with this congenital heart defect.

II. MATERIALS AND METHODS

➢ *Device Design*

The test item under consideration is the patent ductus arteriosus occluder, a self-expandable, mushroom shaped implant. The Patent ductus arteriosus Occluder is a percutaneous, transcatheter device especially designed for closure of normally located Patent ductus arteriosus independent of shape or size. Treatment is feasible in the very young pediatric population. The duration of contact is

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permanent (lasting beyond 30 days). The total dimensions of the occluder are mentioned in the table 1. Sterilization methods include Ethylene Oxide (ETO).

Intended Use: The Patent Ductus Arteriosus Occluder is a percutaneous, transcatheter device especially designed for closure of normally located patent ductus arteriosus independent of shape or size. Treatment is feasible in the very young pediatric population.

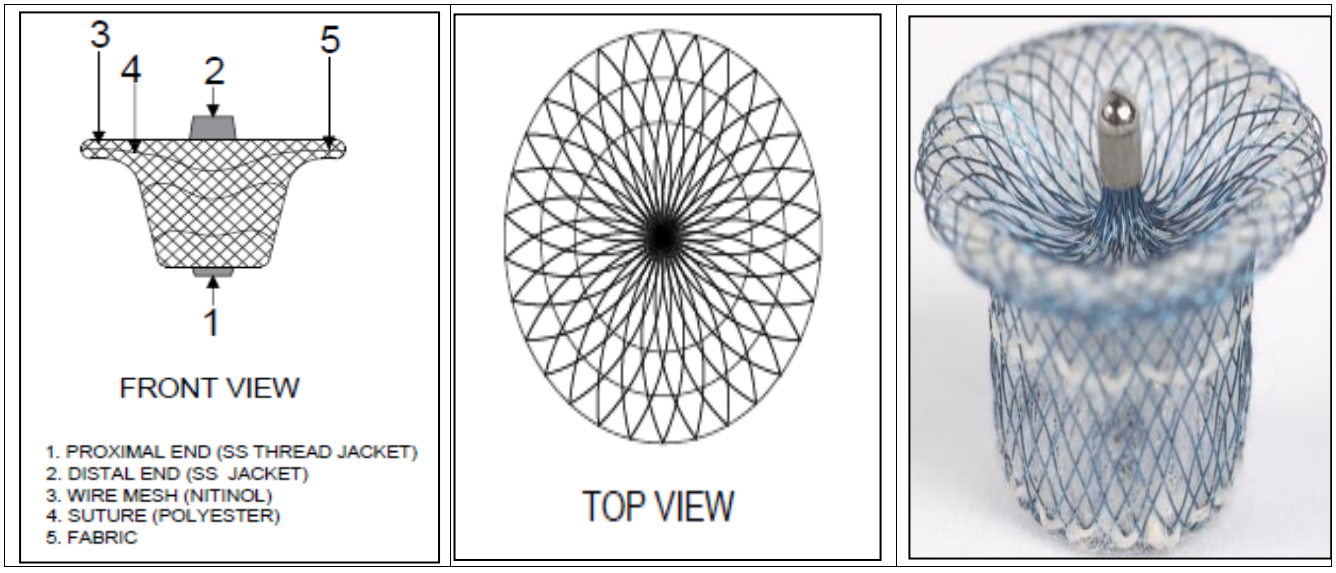
The test item used for the study was floret™ Patent Ductus Arteriosus Occluder manufactured at Meril Life Sciences Pvt. Ltd. The entire available size matrixes are mentioned in the Table 1. From the below mentioned size matrix, occluder having 6-8mm waist size was used to conduct the pre-clinical study to evaluate safety and efficacy of the device.

➢ *Size Matrix*

Table 1: Size matrix of Device

Diameter at Pulmonary Artery (mm)	Diameter of Device (mm)	Retention Skirt (mm)	Length (L) (mm)	Min. Sheath Recommended (F)
4	6	10	7	6
6	8	12	7	6
8	10	14	7	7
10	12	16	7	8
12	14	20	7	8
14	16	22	8	8
16	18	24	8	9
18	20	26	8	10
20	22	28	8	12
22	24	30	8	12

➢ *Product Image*



(A) (B) (C)

Fig 1: Patent Ductus Arteriosus (PDA) Occluder Device
A. Schematic diagram of PDA Occluder (Front View),
B. Side Schematic diagram of PDA Occluder (Top View),
C. Actual Image of PDA Occluder

The Patent ductus arteriosus Occluder is a self-expandable, mushroom shaped implant made from a Nitinol wire mesh. A retention skirt assures secure positioning in the mouth of the Patent Ductus Arteriosus. The communication is closed by PET Patches sewn into the device and some thrombus they induce inside.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24NOV812

➢ *Device Component Description*

Table 2: Device Components Description

Sr. No.	Parameters	Specifications
Implant		
1.	Implant Material	Nitinol Wire
2.	Fabric	PET Fabric
3.	Jacket	Stainless steel
4.	Suture	Polyester Braided
5.	Distal End	Stainless Steel
6.	Proximal End	Stainless Steel
7.	Braided angle	110°-130°
8.	Wire Diameter	70-150 (micron)
Delivery system		
1	Delivery System	An Over the Wire Delivery System
2	Delivery System Usable length	85.6 cm
3	Guide wire compatibility	0.035”
4	Sheath Size (F)	7 to 14 F

➢ *Materials Required*

- Introducer Sheath
- 2-3 Syringes
- Normal heparinised saline (HepNS)
- 0.035’’ diameter guide wire
- Contrast diluted 1:1 with normal saline
- Inflation device
- Three-way stopcock
- Guide Wire Introducer

➢ *Medication Details*

Animals were kept on anticoagulant treatment, Aspirin 300 mg/animal and clopidogrel 75 mg/animal (PO) at least 1days prior to the procedural day, prior to implantation of test item and continued from day 1 to follow up day with reduced dose of aspirin i.e.,100 mg/animal. Animal was weighed, anesthetized, instrumented, and monitored using Ketamine 15 mg/kg (IM), Xylazine 2.5 mg/kg (IM), Atropine 0.05mg/kg (IM) Tramadol 2 mg/Kg (IM) followed by inhalation of anesthesia 1-3% through facemask.

Each animal was given the atropine (anticholinergic) 0.05 mg/kg IM to control discharges from the respiratory tract that may block the endotracheal tube placed for the inhalation of anaesthesia.

The animal was prepared and draped for aseptic procedure with proper medications.

Table 3: Animal 01, 02 and 03 Clinical signs (Pain score) observation on Day 0 to Termination Day

Animal Number		**01, 02 & 03**
Sex		**Male**
Day of Observations	Pain Score	Clinical Signs Incidences
Treatment / Experiment Phase (Day 0)	1	3/3
Treatment / Experiment Phase (Day 1 – Day 3)	1	3/3
Treatment / Experiment Phase (Day 4-90)	0	0/3
Treatment / Experiment Phase (Day 91-180)	0	0/2
Treatment / Experiment Phase (Day 181-365)	0	0/1

III. EXPERIMENTAL PROCEDURES

➢ *Fasting:*

The animals underwent an overnight fast with water deprivation prior to the procedure. Post-procedural care involved maintaining the animals nil per os (NPO) for a duration of six hours following their recovery. This protocol was implemented to ensure optimal conditions for the procedures and recovery phases.

➢ *Animal Preparation included:*

The animals were administered anticoagulant treatment consisting of Aspirin at a dosage of 300 mg per animal and Clopidogrel at a dosage of 75 mg per animal (administered orally) at least one day prior to the procedure. This regimen was continued post-implantation of the test item, with the Aspirin dose reduced to 100 mg per animal from day 1 through the follow-up period.

On the day of the procedure, each animal was weighed, anesthetized, instrumented, and monitored. The anesthetic protocol included an intramuscular administration of Ketamine at 15 mg/kg, Xylazine at 2.5 mg/kg, Atropine at 0.05 mg/kg, and Tramadol at 2 mg/kg, followed by the inhalation of anesthesia at a concentration of 1-3% through a facemask. The thigh area was clipped free of hair to facilitate femoral artery access and the application of ECG leads.

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Atropine, at a dose of 0.05 mg/kg IM, was administered to control respiratory tract secretions that might obstruct the endotracheal tube used for the inhalation anesthesia. The animals were then prepared and draped for aseptic procedures, with appropriate medications administered throughout.

IV. EXPERIMENTAL DESIGN OR ANIMAL TRIAL

➢ *DAY 0*

A percutaneous approach using the Seldinger method was employed to insert an 8F sheath into the femoral artery. Activated Clotting Time (ACT) measurements were taken before and after heparinization. The initial bolus dose of heparin was administered at 100 IU/kg (IV/IA), with subsequent doses titrated based on ACT values.

A stiff 260 cm, 0.035" guide wire was used in conjunction with the guide catheter to navigate to the target region in the carotid artery via the femoral approach. The PDA delivery device was then positioned over the guide wire to the proximal portion of the right carotid artery to implant the PDA occluder, as detailed in table 4. Baseline quantitative vascular angiography (QVA) was performed under angiography to determine the appropriate placement of the PDA occluder based on its diameter.

The PDA occluder device was positioned in the proximal carotid artery following confirmation of its location. Post-implant angiography and QVA were conducted to ensure complete occlusion. Follow-up angiography and QVA were performed 60 minutes post-implantation to confirm the occlusion. The animal was recovered post-procedure and monitored until the terminal day.

Table 4: PDA Occluder Device Implantation Matrix

Animal No	Right Carotid Artery
01	6-8 mm (Waist size)
02	6-8 mm (Waist size)
03	6-8 mm (Waist size)

Table 5: Animal 01, 02, 03 ACT Values (Day 0 and Termination Day)

Sr. No.	Animal No.	Day 0		Day 90		Day 180		Day 365	
		Base line	Post heparin	Base line	Post heparin	Base line	Post heparin	Base line	Post heparin
1	01	86	284	92	296	-	-	-	-
2	02	86	289	-	-	94	298	-	-
3	03	81	301	-	-	-	-	92	290

➢ *DAY 90, 180, 365 –*

Follow-up angiography was performed on day 90 for Animal 01, day 180 for Animal 02, and day 365 for Animal 03 to confirm occlusion and assess arterial stenosis. Animals were euthanized following the 90, 180, and 365-day follow-up procedures for the harvesting of the carotid artery. This allowed for in situ photography of the device, as well as gross and histopathological evaluation. Gross necropsy and photography were also conducted, along with radiographic analysis. Weight of the animals is illustrated in table 6. There was no any abnormality in the body weight of the animal. During the study animal's body weight growth appears to follow a normal pattern. Based on the data, body weight increased by 32.70%, 65.70%, and 113.00% at 90, 180, and 365 days, respectively, for animals numbered 01, 02, and 03. This indicates a proportional relationship between body weight gain and time. Given this proportional trend, the expected body weight increase from 90 to 180 days is approximately double the increase at 90 days, or around 65%. Similarly, the increase from 90 to 365 days is nearly 4 times, or approximately 130%. When comparing the observed values to these expectations, we conclude that the body weight gain in these animals is consistent with normal growth, with no abnormalities detected.

Table 6: Animal 01, 02, 03 Body Weights (Day 0 to Day 365)

Animal Number	Day 0	Day 90	Day 180	Day 365
01	42.5 kg	56.4 kg	-	-
02	41.7 kg	-	69.1 kg	-
03	42.3 kg	-	-	90.1 kg

➢ *Monitoring During Procedure: Day 1 to 365 days*

During the procedure, electrocardiogram (ECG), respiration rate, heart rate, and oxygen saturation were continuously monitored.

➢ *Pre-Operative*

Tramadol (2 mg/Kg IM) as an analgesic was administered to the animal once prior to anesthetic induction. Atropine (0.05 mg/Kg, IM) was also given as pre-anesthetic. Animal was sedated with Ketamine (15 mg/Kg, IM), Xylazine (2.5 mg/Kg, IM) subsequently delivering Isoflurane (1-3%) through face mask.

➢ *Intra-Operative and Post-Operative*

Anesthesia was maintained using 1-3% Isoflurane through endotracheal intubation. All medications administered to each study animal during the procedure were recorded in their individual records.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24NOV812

➢ *Observation*

The study monitored animal body weights, clinical signs of illness or distress, and the performance of test items. Body weights were recorded during acclimatization, procedure, and euthanasia. Animals were observed daily for signs of illness, which were promptly reported to the veterinarian and study director, and documented. The performance of the test items included assessing the delivery system's access, handling, and visualization, as well as deployment accuracy, withdrawal ability, and homeostasis. Additionally, it involved evaluating implant position, integrity, and functionality, along with the histology and pathology of explants. Observation during the study regarding the body weight is, it should not decrease. The given data supported the same and there was no any reduction in body weight. (ref table no. 6).

➢ *Pathology*

- *Clinical Pathology*

Blood was collected before the procedure and at euthanasia for hematological and biochemical analysis.

- *Euthanasia*

Animals were euthanized with thiopental sodium at 100 mg/Kg, IV. Death was confirmed by observing the heart/lungs, a systolic ECG, and zero oxygen saturation.

- *Necropsy*

Gross necropsy was performed on all animals. Organs were weighed and examined, with no lesions found in standard organs.

- *Histopathological Evaluation of Implanted Device*

The implanted device along with the carotid artery was harvested for photography, gross, and histopathological evaluation. The harvested PDA occluder device in the artery was flushed with normal saline and stored in 10% formalin. The device was then processed for block preparation using the resin embedding technique with the Technovit 9100 kit. The embedded tissue was sectioned using a Struer's Secotome 60, polished with a Metco polisher, and stained with Hematoxylene & Eosin stain for histological evaluations of endothelialization, luminal stenosis, and thrombosis. Specimens were collected for histology and histomorphometric analysis. Histomorphometry was performed by Leica LAS image analysis software on all sections cut from the specimens to obtain percent luminal stenosis. Lumen diameter and neointima was recorded. Percent area stenosis was calculated. Histomorphometric data was tabulated and macroscopic observation was included.

Original data was collected for each section, and comments on inflammation or other safety parameters were included.

V. RADIOGRAPHY AND ANGIOGRAPHIC FINDINGS

Radiographic images were taken to evaluate the Occluder to PDA Ratio. In Animal 01, the occluder-to-PDA ratio was calculated as the ratio of the occluder diameter (expected post-implant diameter) to the PDA diameter, yielding a ratio of 0.7 (5.86/8.0). Residual shunting was assessed at 60 minutes and on day 90 with results indicating no shunts and complete occlusion of the artery.

For Animal 02, the occluder-to-PDA ratio was also 0.7 (6.07/8.0). Residual shunting was evaluated at 60 minutes and on day 180, showing no shunts and total occlusion of the artery.

In Animal 03, the occluder-to-PDA ratio was slightly lower at 0.6 (4.98/8.0). Residual shunting was monitored at 60 minutes and on day 365, with findings of no shunts and complete occlusion of the artery.

Table 7: Animal 01, 02, 03 of Morphometric Measurement of Medial Area, Neointimal Area and Percent Stenosis Area of Occluder

A. No.	Stent Location	Part of Stent	Luminal Area (µm)						Internal Elastic Lamina (µm)						External Elastic Lamina (µm)						MA (µm)	NA (µm)	S (%)	
			R1	R2	R3	R4	R5	Mean	R1	R2	R3	R4	R5	Mean	R1	R2	R3	R4	R5	Mean				Mean
01	Carotid Artery	Proximal	0.00	0.00	0.00	0.00	0.00	0.00	6453.33	6478.61	7295.67	7235.84	6627.16	6818.12	8622.31	7842.44	8830.06	8067.82	s8033.03	8279.13	1461.01	6818.12	100.00	87.41%
		Mid	2226.3	2288.92	1786.24	2255.18	2056.91	2122.71	5551.87	5519.05	5881.29	5581.92	5557.48	5618.32	6400.53	6290.97	6511.18	6288.73	6558.55	6409.99	791.67	3495.61	62.22	

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24NOV812

		Distal	0.00	0.00	0.00	0.00	0.00	0.00	5355.32	4909.84	5019.18	4992.83	4864.83	5028.40	6566.04	6732.45	6456.22	6600.33	6756.28	6622.26	1593.87	5028.40	100.00	
	Carotid Artery	Proximal	0.00	0.00	0.00	0.00	0.00	0.00	6108.21	6033.52	6401.56	6281.85	6360.74	6237.18	7965.00	7258.35	8042.49	7643.24	7331.17	7648.05	1410.88	6237.18	100	
02		Mid	0.00	0.00	0.00	0.00	0.00	0.00	4775.82	4948.96	46.34	5125.23	4549.19	4806.64	5180.15	5766.25	5464.66	5542.24	5240.65	5438.79	632.15	4806.64	100	87.18%
		Distal	1278.61	1172.75	1205.42	1208.23	1194.82	1211.97	3996.22	2876.93	3009.12	3036.35	2843.25	3152.37	4681.48	4264.96	3938.16	4071.80	3774.21	4146.12	993.75	1940.41	61.55	
03	Carotid Artery	Proximal	0.00	0.00	0.00	0.00	0.00	0.00	8933.65	8458.80	8279.94	8621.69	8570.51	8572.92	9258.76	8668.47	8718.89	8971.77	8988.25	8921.23	348.31	8572.92	100.00	
		Mid	0.00	0.00	0.00	0.00	0.00	0.00	5018.46	8095.09	7477.5	5729.07	7311.76	6726.38	8472.04	7920.22	8045.7	6870.89	6840.91	7629.95	903.58	6726.38	100.00	94.84%
		Distal	835.85	900.19	840.66	826.16	936.18	867.81	5607.98	5595.57	5583.86	5548.47	5686.56	5604.49	6182.47	6143.19	6008.89	6141.83	6100.92	6115.46	510.97	4736.68	84.52	

Key: A. No. = Animal Number; µm = Micrometer; % = Percentage; S = Stenosis; MA = Medial Area; NA = Neointimal Area

➢ *Pathology*

Blood collection was performed on day 0, prior to the procedure, and on the day of euthanasia. The following hematological and biochemical parameters were analyzed: complete blood count (including differential count and reticulocytes), as well as clinical evaluations of LDH, AST, creatinine, creatine kinase, urea, BUN, sodium, potassium, chloride, and calcium. These assessments were conducted both before and after the procedure.

Both baseline and termination day hematological and biochemical blood counts were within normal ranges on day 0 and at subsequent follow-up time points (days 90, 180, and 365). No abnormal findings were observed in the clinical biochemistry results at day 0, and at days 90, 180, and 365.

Table 8: Individual Data of Animal 01 Clinical Chemistry (Day 0 Baseline, Post Procedure and Day 90)

Parameters	Baseline	Post procedure	Day 90
ALB (g/L)	32.4	31.2	29.6
ALP (U/L)	75	105	80
ALT (U/L)	33	33	37
AST (U/L)	31	32	33
Ca (mmol/L)	2.52	2.53	2.52
T.Chol (mmol/L)	2.05	2	1.51
Creat (µmol/L)	164	144	155
GGT (U/L)	71	90	45
Glu (mmol/L)	3.69	1.02	4.88
Pi (mmol/L)	2.66	3.19	2.19
T.Bil (µmol/L)	4.5	6.17	2.98
T.Pro (g/L)	81.4	78.4	64.1
Trig (mmol/L)	0.2	0.27	0.15
BUN (mmol/L)	2.94	2.96	6.88
Sodium (mmol/L	144.1	143.2	143.7

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24NOV812

Potassium (mmol/L)	3.63	3.92	3.39
Chloride (mmol/L)	104.3	102.7	105.6

Table 9: Individual Data of Animal 02 Clinical Chemistry (Day 0 Baseline, Post Procedure and Day 180)

Parameters	Baseline	Post procedure	Day 180
ALB (g/L)	31.3	26.7	36.5
ALP (U/L)	144	134	94
ALT (U/L)	37	31	32
AST (U/L)	50	44	36
Ca (mmol/L)	2.24	2.2	2.45
T.Chol (mmol/L)	1.83	1.61	1.82
Creat (µmol/L)	162	144	198
GGT (U/L)	41	37	84
Glu (mmol/L)	4.11	2.46	3.99
Pi (mmol/L)	2.05	2.45	2.08
T.Bil (µmol/L)	7.93	6.21	3.37
T.Pro (g/L)	64.7	55.8	68.5
Trig (mmol/L)	0.27	0.11	0.32
BUN (mmol/L)	3.12	2.87	5.65
Sodium (mmol/L	130.8	130.3	144.4
Potassium (mmol/L)	3.6	3.78	3.38
Chloride (mmol/L)	88	94.2	104.1

Table 10: Individual data of Animal 03 Clinical

Parameters	Baseline	Post procedure	Day 180
ALB (g/L)	29.4	27.3	30.8
ALP (U/L)	95	97	33
ALT (U/L)	70	66	63
AST (U/L)	37	35	23
Ca (mmol/L)	2.43	2.49	2.3
T.Chol (mmol/L)	1.29	1.22	1.54
Creat (µmol/L)	170	153	131
GGT (U/L)	37	57	21
Glu (mmol/L)	3.83	2.25	4.83
Pi (mmol/L)	2.26	2.57	2.07
T.Bil (µmol/L)	3.44	3.32	2.93
T.Pro (g/L)	66.5	62.4	56.2
Trig (mmol/L)	0.14	0.08	0.09
BUN (mmol/L)	4.78	4.89	4.57
Sodium (mmol/L	142.5	143.1	136.1
Potassium (mmol/L)	3.9	3.92	3.28
Chloride (mmol/L)	105.4	105	89.9

Chemistry (Day 0 Baseline, Post Procedure and Day 365)

Table 11: Individual DATA of Animal 01 Hematology (Day 0 Baseline, Post Procedure and Day 90)

Parameters	Baseline	Post procedure	Day 90
WBC (109/L)	6.87	2.80	5.79
RBC (1012/L)	6.10	7.22	5.71
HGB (g/L)	104	126	98
HCT (L/L)	0.328	0.386	0.3
PLT (109/L)	506	282	341
NEUT (109/L)	27.8	18	27.7
LYMPHO(109/L)	68.1	77.3	66.8
MONO (109/L)	3.4	1	3.4
EOS (109/L)	0.3	2	0.7
BASO (109/L)	0.1	1	0
LUC (%)	0.3	0.6	1.3
RETIC (%)	0.52	0.7	0.42

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24NOV812

PT (Seconds)	19.4	23.1	18.6
APTT (Seconds)	36.9	62	29.8

Table 12: Individual Data of Animal 02 Hematology (Day 0 Baseline, Post Procedure and Day 180)

Parameters	Baseline	Post procedure	Day 180
WBC (109/L)	10.13	6.76	10.31
RBC (1012/L)	6.06	5.56	6.46
HGB (g/L)	92	83	107
HCT (L/L)	0.286	0.261	0.35
PLT (109/L)	478	421	317
NEUT (109/L)	41.8	38.9	33.7
LYMPHO(109/L)	50.8	56.7	58.6
MONO (109/L)	5.3	2.7	4.1
EOS (109/L)	0.8	1.5	1.1
BASO (109/L)	0	0	0.1
LUC (%)	1.3	0.2	2.4
RETIC (%)	1	0.9	0.75
PT (Seconds)	19.4	18.9	9.3
APTT (Seconds)	24	30.2	25.6

Table 13: Individual Data of Animal 03 Hematology (Day 0 Baseline, Post Procedure and Day 365)

Parameters	Baseline	Post procedure	Day 365
WBC (109/L)	12.1	13.9	7.73
RBC (1012/L)	6.27	6.15	5.23
HGB (g/L)	104	103	100
HCT (L/L)	0.321	0.317	0.302
PLT (109/L)	313	236	182
NEUT (109/L)	60.8	66.5	37.8
LYMPHO(109/L)	36.7	31.6	57.8
MONO (109/L)	1.5	0.3	2.1
EOS (109/L)	0.6	1.1	1.1
BASO (109/L)	0	0.2	0.1
LUC (%)	0.4	0.3	1.2
RETIC (%)	0.46	0.6	0.23
PT (Seconds)	19.7	22.1	14.8
APTT (Seconds)	26.8	47.1	23.1

Table 14: Pathology of Animal 01, 02, 03

Animal Number	Sex	Mode of Death	Macroscopic/Gross pathological Observation	
			External	Internal
01	Male	Terminal Sacrifice	NAD	NAD
02	Male	Terminal Sacrifice	NAD	NAD
03	Male	Terminal Sacrifice	NAD	NAD

NAD: No Abnormality Detected

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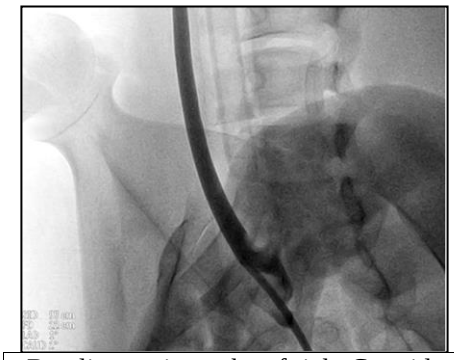
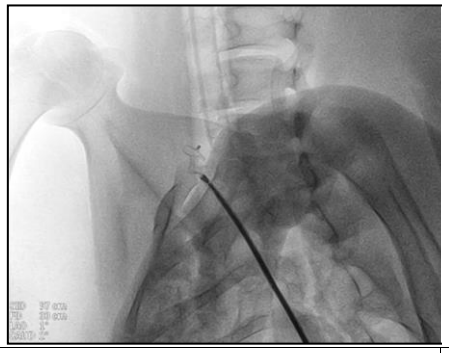
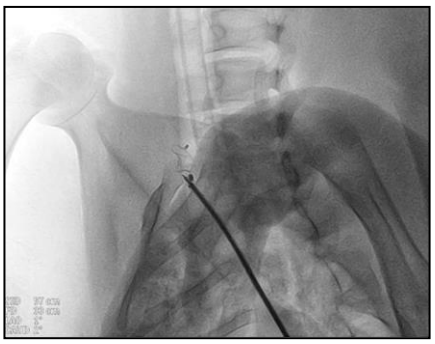
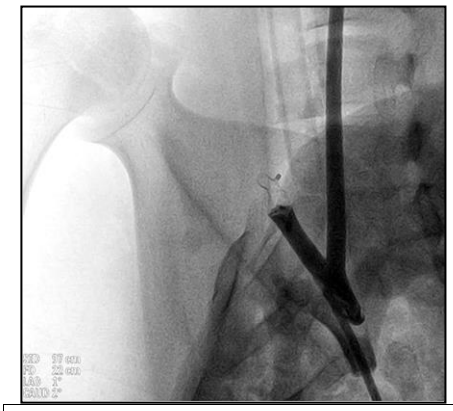
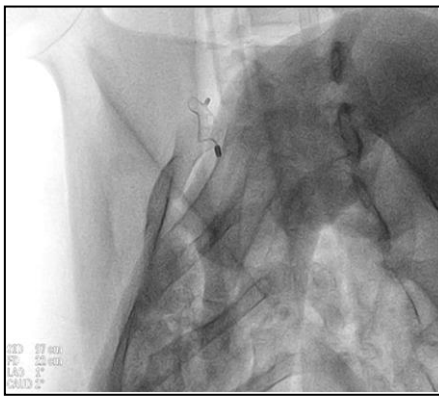
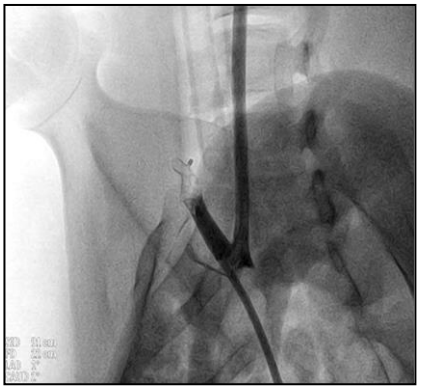
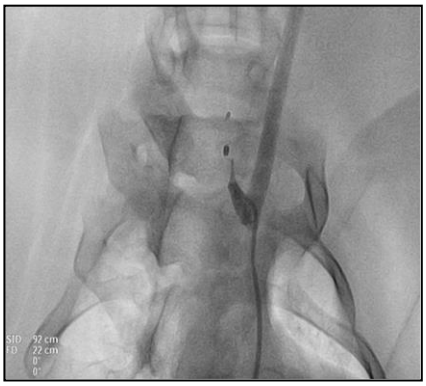
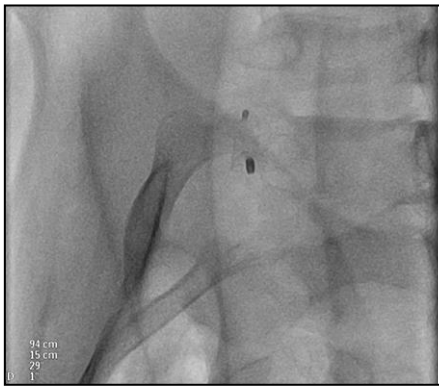
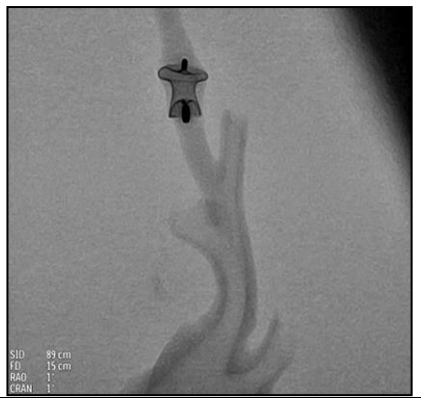
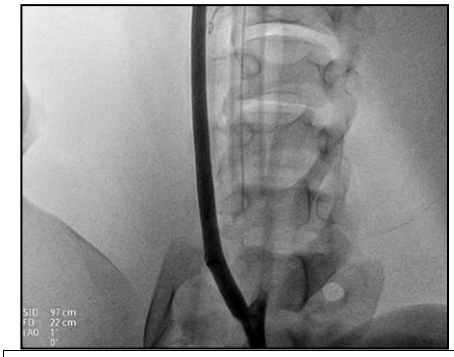
➢ *Fluoroscopy Images*

- *Animal No.01:*

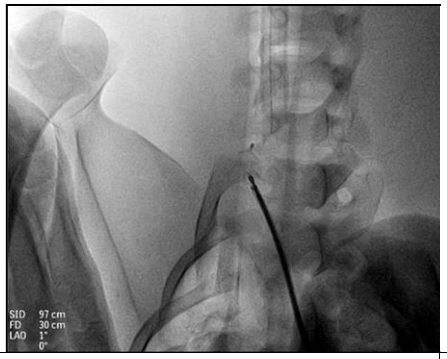
		
Baseline angiography of right Carotid artery	PDA Device positioning in right Carotid artery	PDA Device (with delivery cable) was released in right Carotid artery
		
After released PDA Device post check angio of right Carotid artery	After released PDA Device on Fluoroscopy	After 60 min PDA Device check angio of right Carotid artery
		
PDA Device check angio of right Carotid artery on terminal day 90	Device in Fluoroscopy on terminal day 90	Harvested right Carotid artery fluoroscopy on terminal day 90

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24NOV812

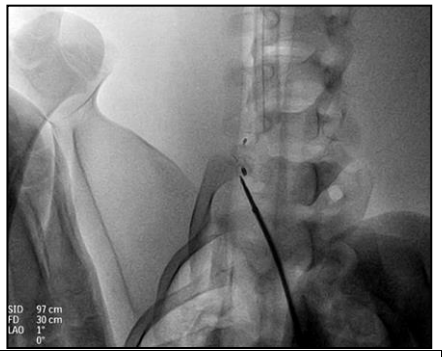
➢ *Animal No. 02:*



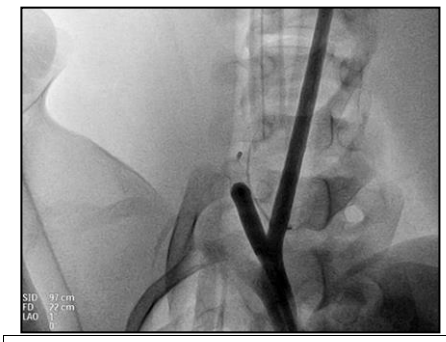
Baseline angiography of right Carotid artery



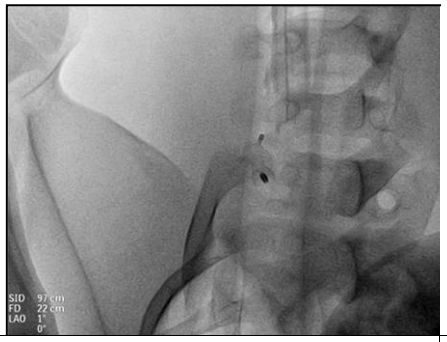
PDA Device positioning in right Carotid artery



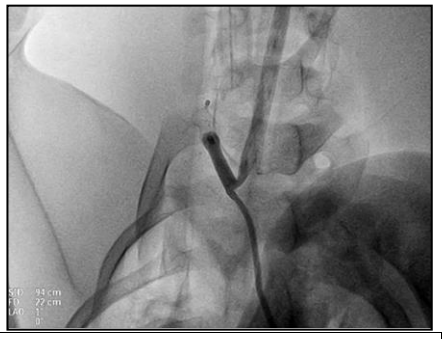
PDA Device (with delivery cable) was released in right Carotid artery



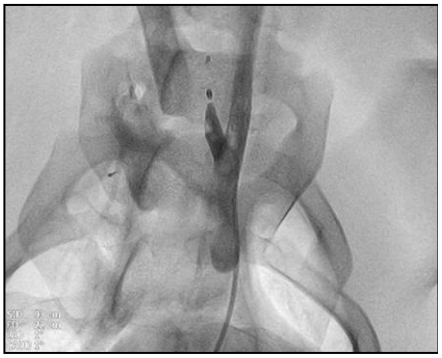
After released PDA Device post check angio of right Carotid artery



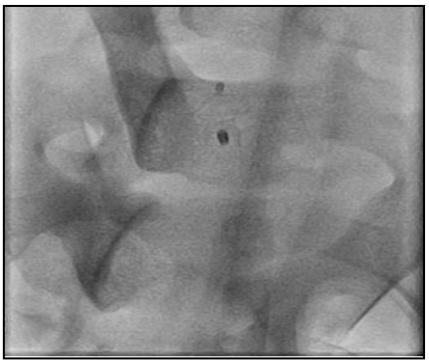
After released PDA Device in Fluoroscopy



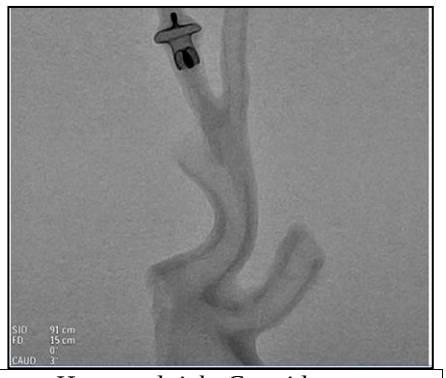
After 60 min PDA Device check angio of right Carotid artery



PDA Device check angio of right Carotid artery on terminal day 180

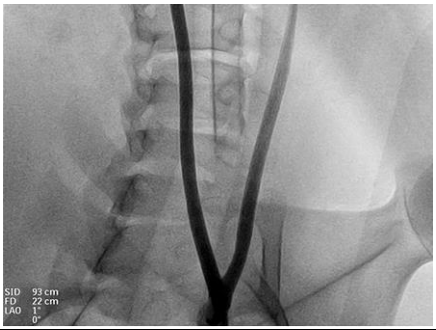


Device in Fluoroscopy on terminal day 180

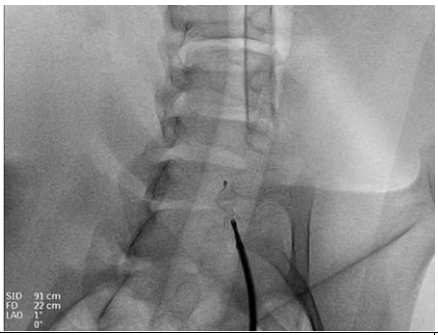


Harvested right Carotid artery fluoroscopy on terminal day 180

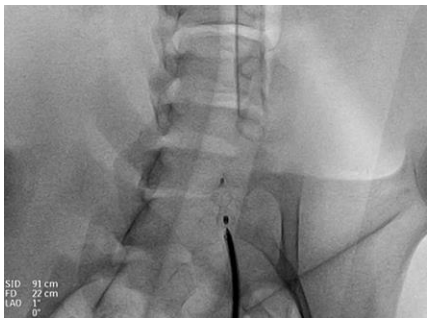
➢ *Animal No. 03:*



Baseline angiography of right Carotid artery



PDA Device positioning in right Carotid artery



PDA Device (with delivery cable) was released in right Carotid artery

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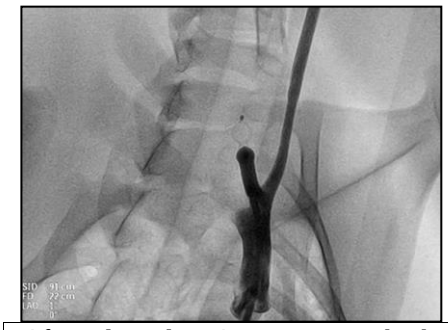
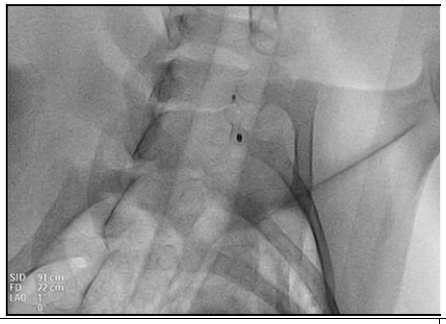
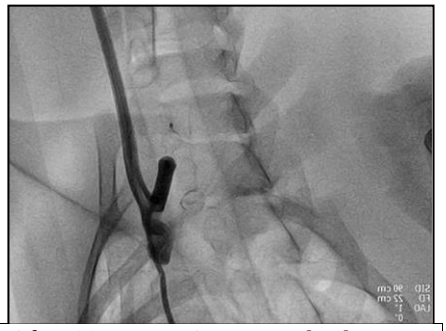


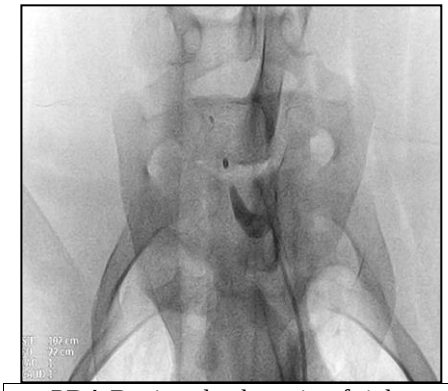
After released PDA Device post check angio of right Carotid artery



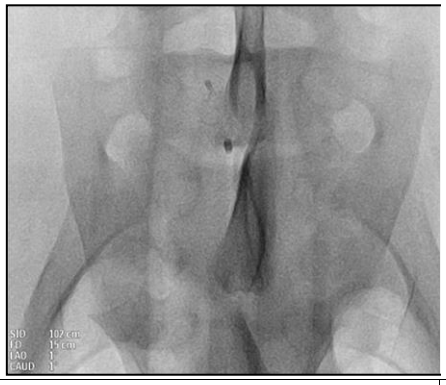
After released PDA Device on Fluoroscopy



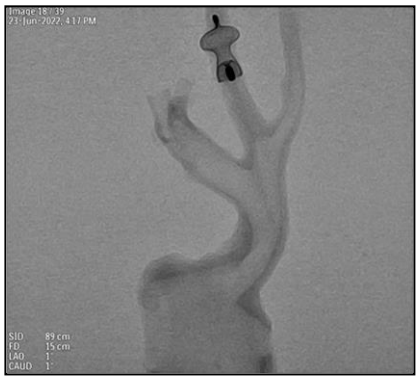
After 60 min PDA Device check angio of right Carotid artery



PDA Device check angio of right Carotid artery on terminal day 365



PDA Device influroscopy on terminal day 365



Fluoroscopy of harvested heart with PFO device in RAO view.

➢ *Necropsy*

At the scheduled sacrifice date, the surviving animals were euthanized via an overdose of thiopental sodium injection. A pathologist conducted a thorough examination for external and internal gross pathological changes. According to the study plan, the PDA occluder artery (carotid artery) was harvested and fixed in 10% neutral buffered formalin. The fixed tissues were then processed for resin embedding and sectioned to a thickness of approximately 100 to 200 microns using a Secotom-60 cutting machine. The thickness was further reduced to an appropriate level using a Bainpol VTD polishing machine. The tissue sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope by the study pathologist to evaluate histopathological lesions and conduct histomorphometry.

External examinations of animals 01, 02, and 03 did not reveal any lesions of pathological significance. Similarly, internal examinations of these animals showed no pathological abnormalities. The right carotid arteries containing the PDA occluder device were collected and flushed with normal saline to assess patency; all arteries with the test item were found to be occluded with no leakage observed.

➢ *Histopathology:*

The histopathology report for animals from 01 to 03 indicates the following findings.

Table 15: Animal 01, 02, 03 of Histopathology Scores for PDA Occluder

A No.	Stent Location	Part of Stent	Inflammation	Carotid artery wall Injury	Smooth muscle cell loss	Fibrin deposition	Endothelial loss	Total Score	Mean Score
1	Carotid Artery	Proximal	3	2	3	3	4	15	13.67
		Mid	3	2	3	3	4	15	
		Distal	3	0	0	4	4	11	
2	Carotid Artery	Proximal	1	1	0	1	4	7	6.33
		Mid	1	1	0	0	4	6	
		Distal	1	1	0	0	4	6	
3	Carotid Artery	Proximal	1	1	0	0	4	6	7.67
		Mid	3	2	0	0	4	9	
		Distal	3	1	0	0	4	8	

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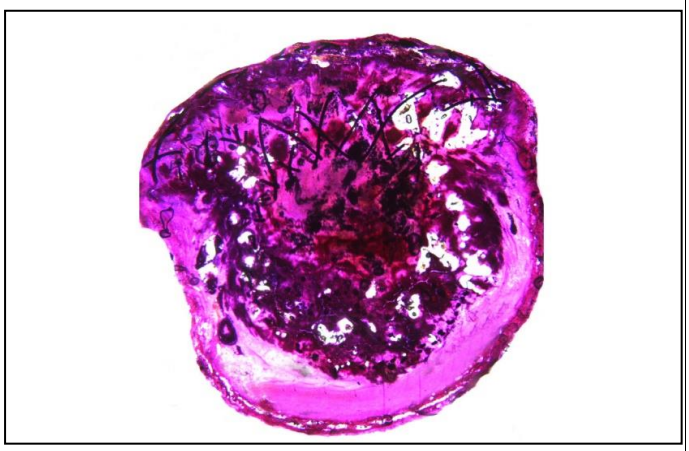
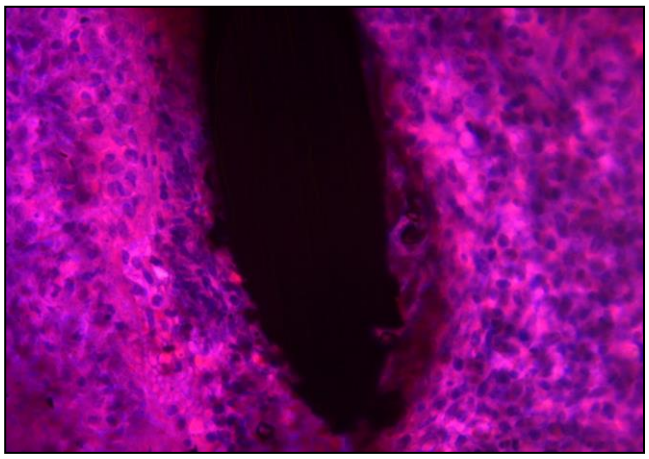
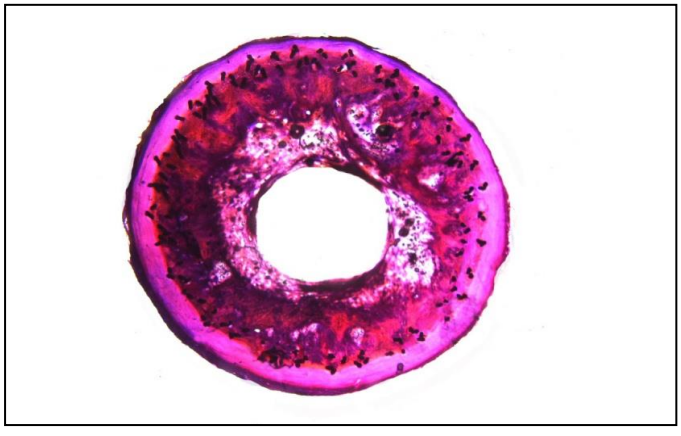
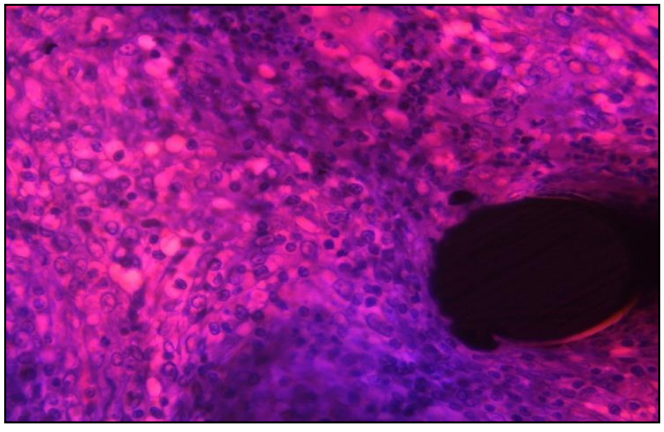
- **Note:** Lesser the score is better histology
- **Keys:** Inflammation (0 - No or very few; 1 - Few; 2 - Many without circumference; 3 - Many with circumference) Carotid artery wall injury (0- No; 1- Perforation of Internal Elastic Lamina; 2- Perforation of Media; 3- Perforation of External Elastic Lamina) Smooth muscle cell loss (0- No; 1- Minimal (< 25%); 2- Mild (25-50%); 3- Moderate (51-75%); 4- Marked loss (>75%) Fibrin deposition (0- no;1- Minimal ; 2- Mild; 3- Moderate; 4- severe) Endothelial cell loss (0- No; 1- minimal (< 25%); 2- mild (25-50%); 3- moderate (51-75%); 4- marked loss (>75%).

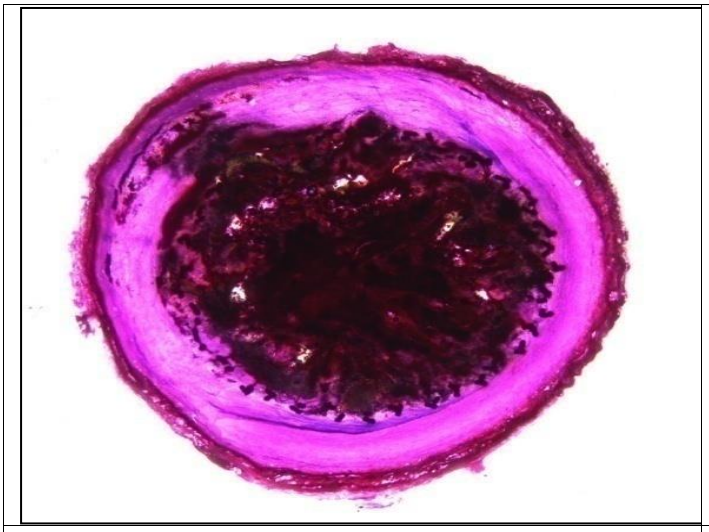
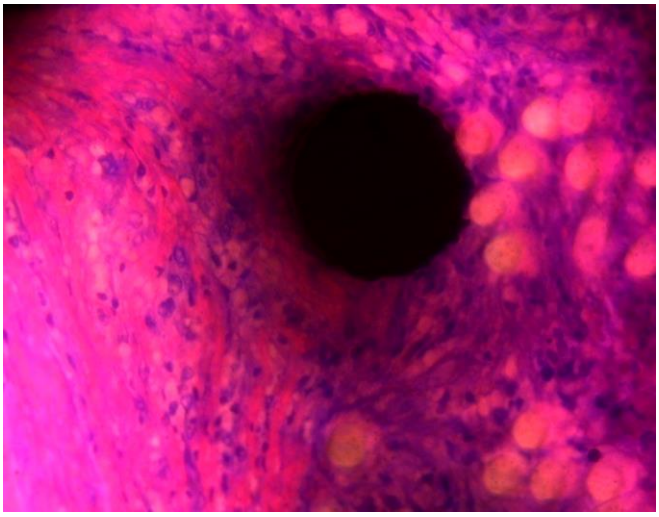
➢ *Animal No.01:*

The PDA Occluder was found to have numerous inflammatory cells with circumference in its proximal, middle, and distal parts. There was perforation of media in the proximal and middle parts, while no Carotid artery wall injury was found in the distal part. Smooth muscle cell loss was moderate in the proximal and middle parts, while fibrin deposition was moderate in the proximal and middle parts, and severe fibrin deposition was observed in the distal part. Endothelial loss was also present throughout the PDA occluder. Histo-morphometric measurements revealed no luminal area in the proximal and distal parts, and a marked increase in neointimal area in the proximal, middle, and distal parts, indicating severe lumen loss and contributing to a mean stenosis area of 87.41%.

Table 16: Histopathological Images of PDA Occlude Animal no. 01 H&E 1.25X and 40X.

H&E 1.25X	**H&E 40X**
	
A1 Proximal, carotid Artery PDA Occluder	A1 Proximal, carotid Artery PDA Occluder
	
A1 Mid, carotid Artery PDA Occluder	A1 Mid, carotid Artery PDA occluder

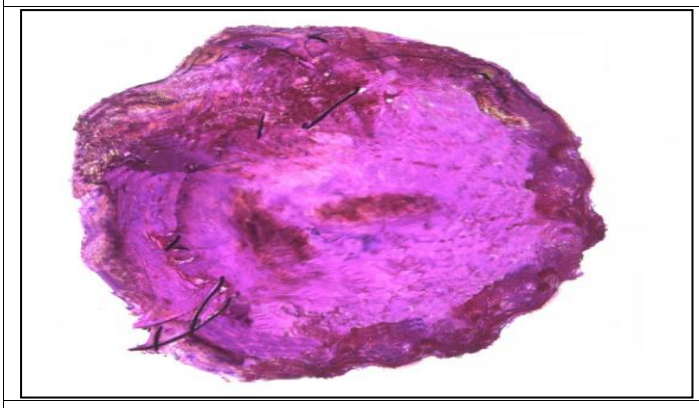
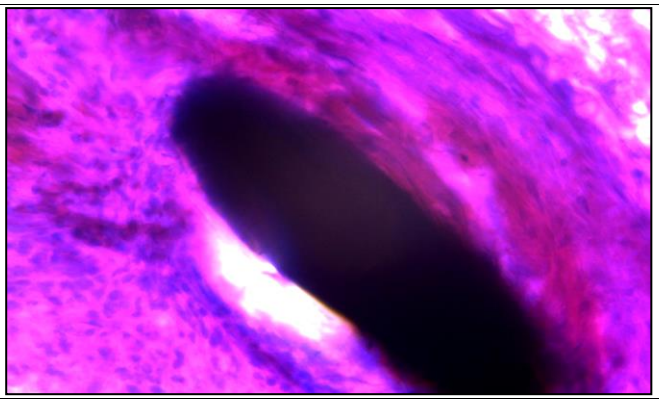
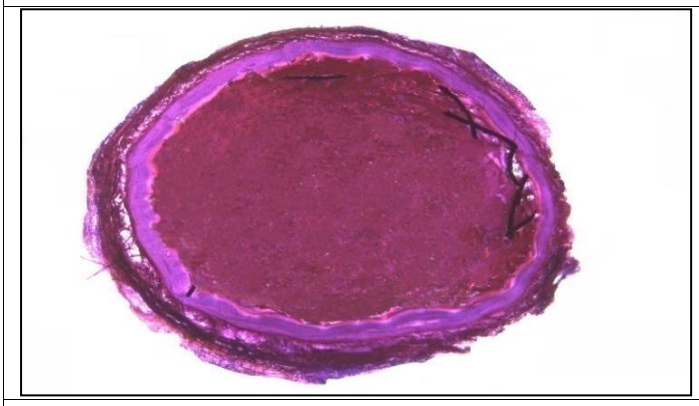
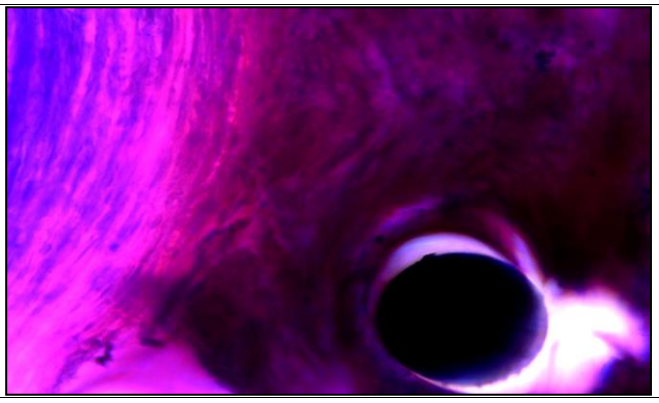
ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24NOV812

	
A1 Distal, carotid Artery PDA Occluder	A1 Distal, carotid Artery PDA Occluder

➢ *Animal No.02:*

The PDA Occluder was found to have few inflammatory cells in its proximal, middle, and distal parts, with a break in the internal elastic lamina. There was no smooth muscle cell loss, minimal fibrin deposition, and marked endothelial loss throughout the PDA occluder. Histo-morphometric measurements revealed no luminal area in the proximal and middle parts, and moderately reduced luminal area in the distal part. The neointimal area increased significantly in the proximal, middle, and distal parts, indicating severe lumen loss and contributing to a mean stenosis area of 87.18%. The stenosis area measured from 100% to 61.55%, with stenosis in the proximal, middle, and distal parts.

Table 17: Histopathological Images of PDA occlude Animal no. 02 H&E 1.25X and 40X.

H&E 1.25X	**H&E 40X**
	
A2 Proximal, carotid Artery PDA Occluder	A2 Proximal, carotid Artery PDA Occluder
	
A2 Mid, carotid Artery PDA Occluder	A2 Mid, carotid Artery PDA Occluder

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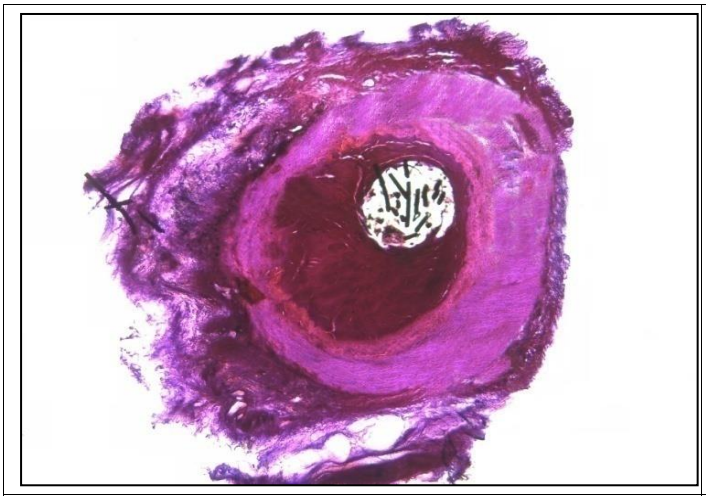
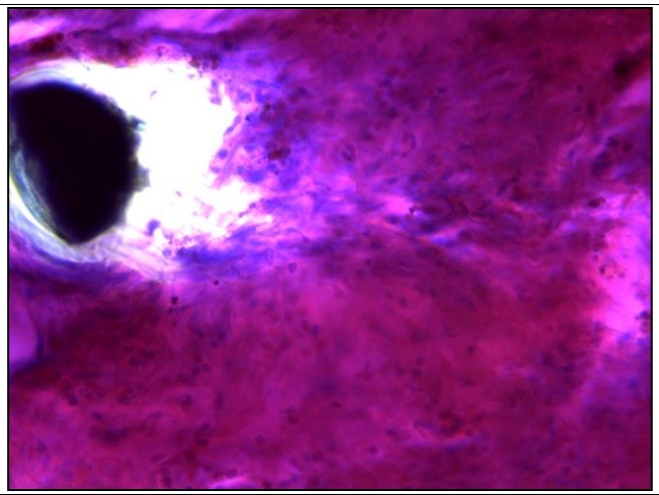
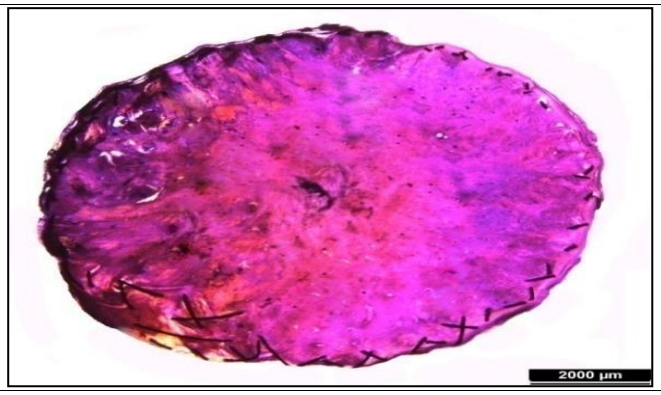
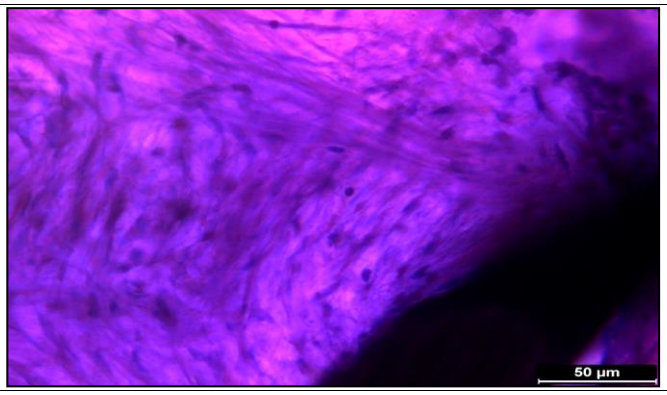
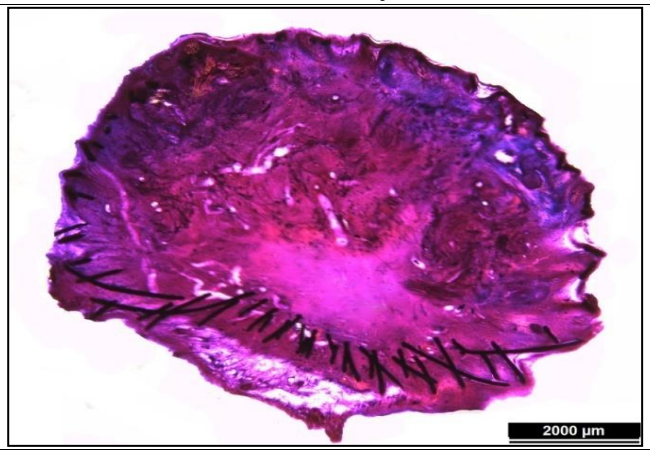
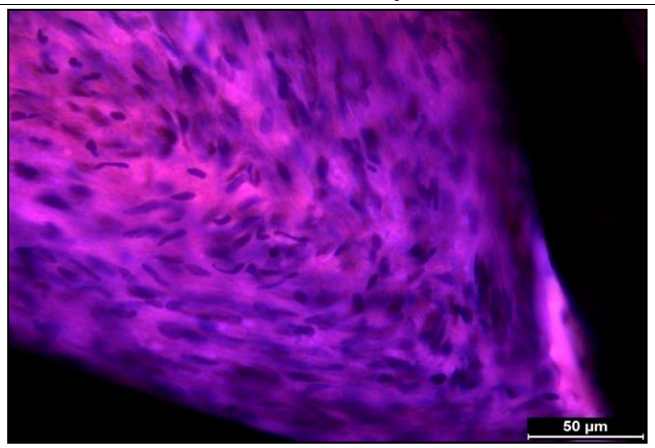
	
A2 Distal, carotid Artery PDA Occluder	A2 Distal, carotid Artery PDA Occluder

➢ *Animal No.03:*

The study examined the histopathology of the PDA Occluder, revealing few inflammatory cells in the proximal part and many cells with circumference in the middle and distal part. There was a break in the internal elastic lamina and media perforation in the middle part. No smooth muscle cell loss was observed in the proximal, middle, and distal parts, and no fibrin deposition was found. Endothelial loss was marked throughout the PDA Occluder. Histo-morphometric measurements revealed no luminal area in the proximal and middle parts, but a moderately reduced luminal area in the distal part. The neointimal area increased significantly in the proximal, middle, and distal parts, indicating severe lumen loss and contributing to a mean stenosis area of 94.84%.

Table 18: Histopathological Images of PDA occlude Animal no. 03 H&E 1.25X and 40X

H&E 1.25X	**H&E 40X**
	
A3 Proximal, carotid Artery PDA Occluder	A3 Proximal, carotid Artery PDA Occluder
	
A3 Mid, carotid Artery PDA Occluder	A3 Mid, carotid Artery PDA Occluder

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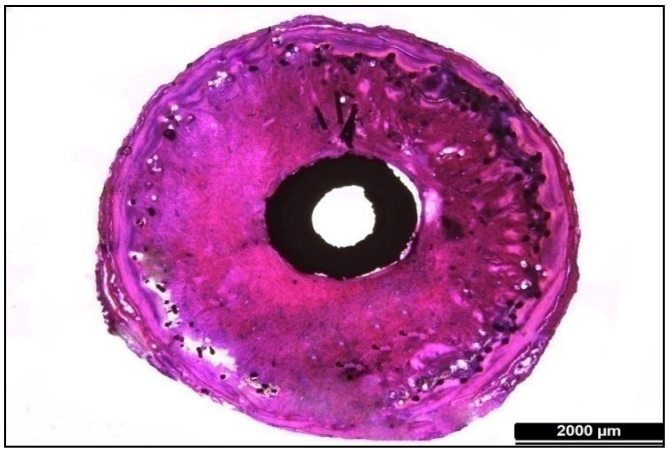
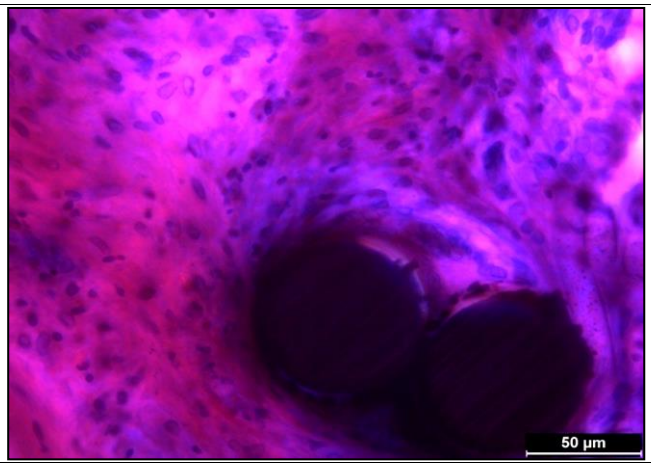


A3 Distal, carotid Artery PDA Occluder



A3 Distal, carotid Artery PDA Occluder

VI. RESULT

Throughout the year-long study (Day 0–365), the animals were closely monitored for signs of ill health. Observations showed that all animals exhibited normal behaviors, such as eating, drinking, defecating, and urinating. The animals appeared bright, alert, and responsive, indicating no adverse health effects from the procedures or devices used.

Body weights were recorded at specified intervals, and no instances of weight reduction were noted, suggesting good overall health and normal growth. The delivery system for the PDA occluder device successfully accessed the target site (carotid artery) from the femoral artery. The system was handled and visualized efficiently, ensuring functional hemostasis. Deployment accuracy and efficacy were high, with no device migration or leakage observed. Angiographic confirmation post-implantation demonstrated the correct position and integrity of the device, with satisfactory expansion and ease of deployment. Histopathological assessments confirmed effective endothelialization and no significant inflammatory or thrombotic responses.

Morbidity or mortality was not observed among the animals throughout the experimental phase. Fluoroscopic images taken at intervals (Day 0, 90, 180, and 365) confirmed the device's stable position without migration. Radiographic evaluations supported these findings, showing consistent device placement. Blood samples collected on Day 0 and at euthanasia revealed that all hematology and biochemistry parameters remained within normal ranges. No abnormal findings were observed, suggesting the absence of adverse systemic effects from the device.

Necropsies conducted at scheduled sacrifice dates revealed no external or internal pathological changes in animals 01, 02, and 03. Carotid arteries with the PDA occluder were processed and examined histologically. Animal 01 exhibited inflammatory cells and smooth muscle cell loss primarily in the proximal and middle sections, with significant fibrin deposition and endothelial loss. The stenosis area averaged 87.41%. Animal 02 had fewer inflammatory cells and minimal fibrin deposition, with an average stenosis area of 87.18%. Animal 03 showed a similar pattern, with an average stenosis area of 94.84%. These findings indicate varying degrees of histopathological changes and stenosis, particularly in the proximal and middle sections of the occluder.

VII. DISCUSSION

In this study, the Patent ductus arteriosus (PDA) Occluder was successfully implanted in a porcine carotid artery model, meeting all key criteria including proper deployment and animal survival throughout the procedure and post-procedural period. The device exhibited easy trackability and visualization, with no cranial artery flow above it post-deployment. Gross necropsy and histopathological assessments at Days 90, 180, and 365 showed good endothelialization and a bulged carotid artery, indicating effective integration with arterial tissue. The occluder achieved complete arterial occlusion at all follow-up points without migration. All animals survived with the device intact at the target site, demonstrating its reliability and safety. The deployment process was straightforward, with easy withdrawal post-deployment. Overall, the PDA occluder demonstrated promising clinical potential for occluding patent ductus arteriosus, showing reliable performance, safety, and efficacy in the porcine model. These findings support its use in interventional cardiology for precise vascular occlusion.

These findings collectively demonstrate the device's strong biocompatibility, durability, and functionality, indicating its suitability for clinical use in managing conditions related to Patent ductus arteriosus in humans.

VIII. CONCLUSION

The study successfully demonstrated the efficacy and safety of the Patent ductus arteriosus (PDA) occluder in a porcine carotid artery model over a one-year period. The animals showed no signs of ill health, exhibiting normal behaviors such as eating, drinking, defecating, and urinating, with regular monitoring of body weight indicating healthy growth and no weight loss, confirming the absence of adverse effects from the procedure or device. The PDA occluder was deployed with high accuracy and effectiveness, achieving its

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target site from the femoral artery to the carotid artery without complications, showing excellent trackability and visualization for efficient hemostasis and proper deployment. Post-implantation angiographic and fluoroscopic evaluations confirmed stable positioning and device integrity, with no migration or leakage observed, corroborated by radiographic assessments. Histopathological assessments revealed effective endothelialization and minimal inflammatory or thrombotic responses, indicating good biocompatibility. Blood sample analyses showed normal hematology and biochemistry parameters, suggesting no adverse systemic effects, while necropsy and histological examinations confirmed no pathological changes except for varying degrees of inflammatory responses and stenosis in the occluder's proximal and middle sections. The PDA occluder achieved complete arterial occlusion at all follow-up points, demonstrating its reliability and ease of use, and consistent animal survival underscored its safety. These findings suggest that the PDA occluder holds significant clinical potential for managing Patent ductus arteriosus in humans, supporting its further development and clinical application for precise vascular occlusion. (IF WE HAVE ANY PATENT OR PUBLICATION ON THIS TYPE OF OCCLUDER DEVICE, WE CAN ADD THIS AS EXTENSION OF THE STUDY WITH THE REFERENCE)

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