

MACHINE LEARNING ANALYSIS ON THE IMPACT OF BISPHENOL A (BPA) EXPOSURE ON SKELETAL DEVELOPMENT

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ABSTRACT

Bisphenol A is a synthetic compound commonly found in human consumer products. This hazardous material possesses estrogenic properties that might negatively impact human health, especially during crucial stages of growth and development. The present study aims to evaluate the effect of BPA on bone cell differentiation within chick embryos. The chick embryos used in this paper serve as an appropriate model system for investigating skeletal development as the potential adverse impact of BPA exposure on it. Various concentrations of BPA were delivered to chick embryos at different time points. Micro-CT images of chick embryos bones were used to identify the anomalies in bone development induced by the exposure to Bisphenol A. This study has found a prominent abnormality in bone morphology, such as fractures, disruptions, and changes in bone density above exposure to BPA. The skeletal disturbances among BPA-exposed chick embryos, especially on the 18th day after development, in case of high doses of the compound, were most notable. The VGG19 model used in present study demonstrated decent accuracy in “the classification of anomalies induced by BPA exposure”. Overall, it was possible to suggest that BPA exposure on varied levels and time points of chick embryos development was able to be detected by the model with a high level of accuracy. Moreover, the results of this study emphasize the hazardous nature of BPA exposure on human health.

KEYWORDS: Bisphenol A, BPA, skeletal development, endocrine-disrupting chemical, bone morphology

1. INTRODUCTION

Bisphenol A, an organic synthetic compound used in most industrial processes for manufacturing hard plastic bottles and food packaging materials, has gained public concern of potential adverse effects associated with human health. Being a well-known estrogenic compound and a member of the endocrine disrupter chemical [1], [2], BPA was considered a health hazard in terms of its influence on many physiological human processes, especially during crucial developmental periods. Skeletal development concerns became significant for scientists because of the ubiquity of BPA-containing materials that might affect both human health and animal organisms. BPA exposure was linked to such health complications as developmental abnormalities, hormonal changes, reproductive system diseases, and rising chronic disease rates in humans. Infants, children, and embryos are considered the most vulnerable for BPA exposure because its manifestation during critical periods of growth and development might result in severe skeletal complications. Thus, an understanding of its mechanisms of action is crucial for public policy implications, as early exposure might have lifelong damaging effects [3]–[6]. The research concerns the investigation of BPA effects on bone cell differentiation in chick embryos, which is considered a reliable model organism for bone development examination. The administration of different BPA concentration will allow monitoring skeletal development at different stages and understanding an integrated toxicity component. In this investigation, we utilize microcomputed tomography to obtain the chicken embryo bones' imaging data to specify any key changes in bone morphology and density concerning the time post yolk that skeleton has been forming [7]–[9]. The experiment also applies machine learning approach known as VGG19 model to analyze the obtained micro-CT images with the aim to detect BPA toxicity effects on bone formation.

2. LITERATURE REVIEW

Bisphenol A is one of the most widely used synthetic compounds that has a variety of applications across industries, mainly in the production of polycarbonate plastics and epoxy resins. The efficiency and low-cost production make it a

popular tool in the production of a variety of consumer items: food and drinking bottles, receipt papers, dental sealants, medical devices, to name a few [10]. Bisphenol A has risen to prominence in the past few years because of the potential negative health effects that the compound is believed to possess on humans, especially estrogenicity and categorization as an endocrine disrupting chemical [11], [12]. As endocrine disruptors, EDCs have the capacity to interfere with the hormone system by way of mimicry or other means of subverting normal human physiology. BPA's capacity to serve as an EDC makes a potentially dangerous chemical to humans and lies in the danger it poses during the critical stages of development in the human lifecycle, specifically fetal development and early childhood.

Considering the potential harmful influences of BPA, many investigators have paid considerable attention to this topic. Findings of various research of the topic suggest that a variety of health problems is associated with the impact of BPA. Some of the negative consequences encompass developmental abnormalities and hormonal disbalance, reproductive malfunctions, and a greater predisposition to some chronic diseases. The reproductive system is deemed to be particularly vulnerable to the effects of BPA as it disrupts the mechanisms of hormone signaling, inhibits proper ovary function, and reduces sperm quality [13], [14]. Moreover, it is assumed that BPA is a cause for the most common metabolic disorders, like obesity, insulin resistance, and type 2 diabetes, as well as cardiologic diseases, neurodevelopmental issues, and types of cancers. The most sensitive populations affected by potential exposure are infants, children, and the developing prenatally, as their systems are impaired at the most critical stages of development, and, thus, are at high risk of acquiring the affected health conditions even long after their exposure. [15].

The mechanisms of BPA toxicity are complex and difficult and are primarily based on the interactions of this substance with many cellular and molecular pathways. The primary mechanism of toxin's action is the ability of BPA to bond with estrogenic receptors and to inhibit estrogen in the organism. BPA binds to both α and β estrogen receptors, acts as agonistic molecule in the tissues and activates estrogen-responsive genes, and affects the spectrum of gene expression [16]. Additionally, BPA can affect other signaling pathways, including aryl hydrocarbon receptor pathway, peroxisome proliferator-activated receptor pathway, and thyroid hormone receptor. Through these pathways, BPA can affect the signaling of different hormones in the body, disrupting cellular function and causing development of a variety of health concerns. Finally, BPA has been found to exert its effects through epigenetic mechanisms, affecting the patterns of DNA methylation, histone modifications, and microRNA expression, ultimately affecting the expression of the genes and function of the cells. The mechanisms of BPA toxicity are therefore very complicated and involve multiple molecular targets and pathways [17]. A relatively large body of evidence points to the fact that Bisphenol A being known for exhibiting endocrine disrupting characteristics has a potential to negatively affect the development of health of skeletal system leading to structural abnormalities, changes in bone density and bone turnover [18]. It should be noted that the majority of findings on this matter comes from animal studies. In particular, BPA was shown to cause abnormalities in the morphology of bone tissue, disturb mineralization and affect the markers of bone turnover [19]. For instance, some research on rodents indicates that prenatal and neonatal exposure to BPA results in decreased bone mineral density, altered bone matrix quality, architecture and homeostasis and elevated risk of fractures at older age [20]. Moreover, in vitro research that utilized cell culture models identified that BPA disrupts the differentiation of osteoblasts into mature bone cells, blocks mineralization and increases activity of osteoclasts, which degrades bone tissue. Lastly, the findings of human epidemiological studies suggest that there is a link between BPA exposure and some health outcomes related to skeletal development, including decreased bone mineral density, risk of osteoporosis and fractures at older age. However, the evidence on its role in skeletal development in human is very incomplete and the available data is often inconsistent. Overall, it is necessary to conduct more studies to identify the processes that may be disrupted by BPA and the causal relationships between exposure to this substance and health outcomes[21].

There are several gaps and differences in our understanding of BPA's impact on people's health. While there are some data regarding the toxic skeletal effects of BPA, most studies are limited when discussing human impacts. It is essential to mention that the majority of existing studies analyze animals and in vitro experiments, and there are relatively few human-based researches to speak of. Thus, the skeletal toxicity of BPA remains relatively vague, and it is unknown at what doses or periods of exposure people or other animals may experience hazards to their bones. There are also still relatively few longitudinal studies into the lasting effects of BPA exposure to skeletal development and the associations with other factors that may facilitate or impair our health. Given the closeness and complexity of contact shown to BPA, exposure's impact on human bones will require several types of studies to determine, such as studies, experimental models, and mechanisms of BPA action. Such steps would make it possible for people to build an effective and systematic policy to reduce BPA exposure and limit the number of people affected globally on a regular basis [8], [11], [14], [22].

Studies exploring the effects of BPA on the skeletal development use a wide range of research methodology and technique. Researchers using animal models have exposed both mice and rats to BPA, both prenatally and postnatally. The skeletal assessment methods employed in these studies include the following: bone histomorphometry; dual-energy X-ray absorptiometry, microcomputed tomography and mechanical testing. In vitro studies have used different cell types, including osteoblasts and osteoclasts, to study the effect of BPA on bone resorption, mineralization and differentiation. Human studies include both cohort and case-control studies and cross-sectional studies. These studies have examined the potential effects of BPA exposure on a person's bone mineral density. In addition to these studies, some researchers have also studied the association of BPA with osteoporosis and fractures.

3. METHODOLOGY

Bisphenol A is a compound endowed with estrogenic hormonelike properties. It is also known as one of the Endocrine Disrupting Chemicals, posing serious health implications, especially when people reach critical development stages such as fetal, infant and childhood development. In this study, it was discovered that BPA, dosed at 0.01 $\mu\text{g/kg}$ on the 5, 10, 14

and 18 days of incubation has a statistically significant impact on chick embryos bone development. For conducting the search and the following experiment, 20 chick embryos were used following the principle of maximum control, i.e. 10 chick embryos were dosed with BPA, and 10 chick embryos were used as a control group for normally developing embryo in the incubation process only no BPA dose was provided. The experiment was divided into several intervals in order to carefully administrate the BPA into the chick embryos separating these experiments by sufficient time in order not to disrupt the usual incubating process. Moreover, the dosing procedure was controlled according to the feeder and protocols of administrating BPA. Each incubating chick embryo in the control group was handled according to the same procedures as in the BPA experimental group, but they were not provided with BPA dose in the solution.

In order to assess the impact that BPA had on chick embryo's bone structure development, Micro X-ray Computed Tomography (μ XCT) was used to gather information about any changes in the bone structures of a chick embryo being the veritable morphological correlate in studying the internal and external bone skull morphology changes, such as fractures, deformation and changing of bone density. Finally, the influx of Machine Learning models that were previously calibrated with the historical chick embryo experimental data, containing multiple, diverse attributes of bones' length, width, density and many more, was also used in the data analysis, including building the models, and selection of the most essential attributes, developing the model, and finally, validating the model. The purpose of the model application was to predict the outcome of the BPA dose and its role in causing the underlying mechanisms leading to the health problems for the chick embryos at this stage. The methodology of the research is shown in figure 1.

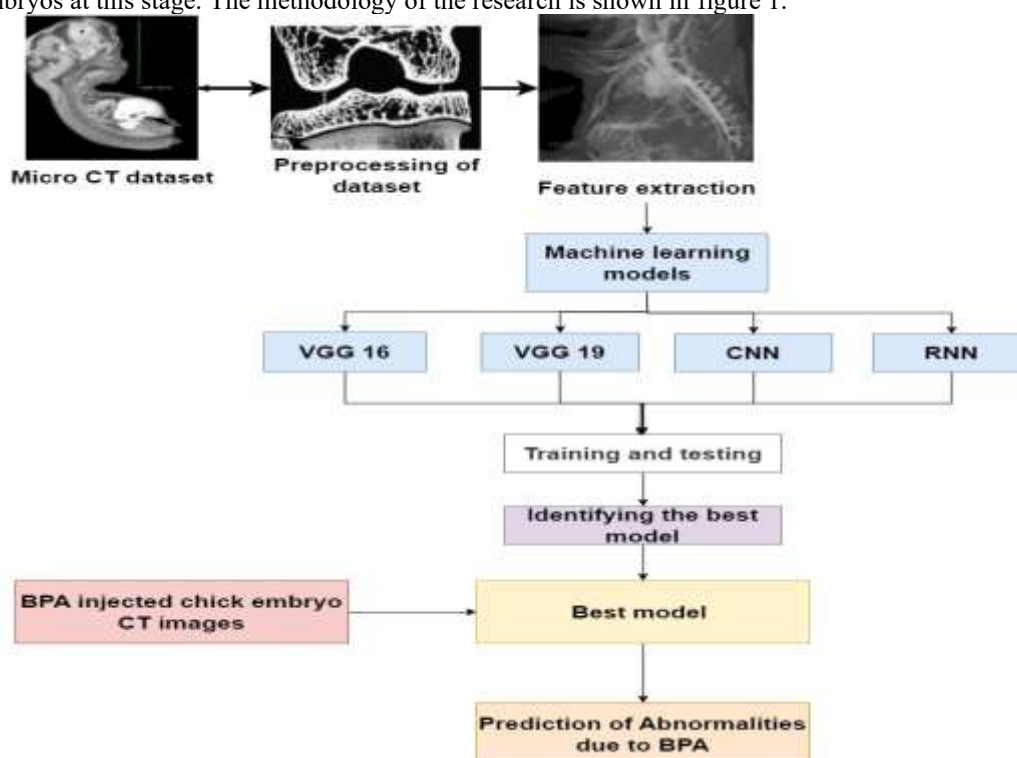


Fig. 1. Architecture of the proposed research

Dataset

To conduct this research, at first, an image dataset was created. The initial dataset contained 300 images that were found on online images repositories. This dataset was used as a sample to get a deep understanding of chick embryo skeletal development. Then, to create a specific dataset related to the differences in chick embryo bone growth under normal conditions and the influence of Bisphenol A, a chemical that was used on different levels in everyday life, some measurements were done. In other words, the chick embryo bones were examined by the Microcomputed Tomography to create a more detailed dataset of the chick embryo skeletal development in terms of the two conditions. In such a way, a whole dataset of 550 images at different stages was built. It is important to specify that the work with chick embryos was ethical and did not violate any ethical rules concerning chick embryo research making the work in line with the established standards and protocols.

The dataset was divided into 70% for training the machine learning models and 30% for testing. Therefore, 385 images of the chick embryo skeletal development at different stages were used for training, and 165 other images were used to test how the models can be used to detect the above-mentioned differences at different stages. The division of the dataset into training and testing datasets was essential because it preserves the clearness of the division into two types of datasets, and at the same time, it provides the opportunity to state the highly relevant and credible results of the conducted research. One of the largest advantages was that the models were trained using 70% of the universal dataset and tested using 30% of the sample that presented the universal dataset. As a result, the DL models used in this research were numerous: different types of CNNs, among which there were VGG16 and VGG19; RNNs to catch the temporal dependencies in the dataset.

Machine learning models

For this research, by utilizing the dataset, a number of machine learning (ML) models were introduced to explore and understand the impacts of Bisphenol A exposure on the chick embryo skeletal development. Novel and efficient architectural solution to this problem is the application of Convolutional Neural Networks to acquire valuable and significant features of the images obtained. This method is the best for image recognition as it is able to learn the hierarchical representation of the input data. The dataset used in this research is related to the chick embryo skeletal images, so the application of the popular VGG16 and VGG19 architectures to process it was made. The VGG networks consist of several convolutional layers that are followed by the fully connected ones, so the resulting network is able to detect the complex patterns and secretaries in the images. Therefore, the purpose of this study was to identify the slight differences between the skeletal images of the chick embryos treated with BPA and the control group images and define the abnormalities caused by the BPA exposure.

Besides, the application of Recurrent Neural Networks was made to analyze the dataset and help determine the skeletal development trajectories. The RNN are good for handling the sequences of data, that is very helpful while trying to track the growth of the chick embryo bone in a week, the RNN also have the ability to eliminate the spatial relationships, which is especially useful for this image-related project. Therefore, the use of this network makes it possible to discover the dynamic nature of the skeletal development and its trends with the view of detecting the abnormalities related to the BPA treatment. The use of RNN enables the models to analyze the static data and the information requiring memory.

Preprocessing of images

A technical approach was taken in the research to preprocess and prepare the dataset for subsequent analysis by implementing efforts to improve the quality of data, standardize features, and add to the diversity of the dataset. Each step in the preprocessing pipeline was intended for achieving specific goals associated with the dataset. The procedure, therefore, was aimed at fostering more valuable and enjoyable analysis of the effects of BPA exposure on chick embryo skeletal development. The first step in the preprocessing pipeline was data cleaning which was intended to make sure there were no inconsistencies, mistakes, or missing values in the dataset. The step required a meticulous approach and allowed to develop and implement a range of validation procedures to ensure the suitable quality of all collected images and associated metadata in relation to which analysis was conducted. All corrupted images, errors in labeling samples, and missing pieces of metadata were addressed and rigorously checked to be excluded from the following steps to ensure that the dataset was clean, and, thus, the results were less exposed to potential biases. The next set of procedures was related to normalization and standardization. While normalization allowed to mitigate variations inherent in the task, standardization techniques allowed controlling any possible differences in feature scaling and scaling all of the features in relation to all of the images to zero mean and unit variance decreasing the severity of convergence of the machine learning model and enhancing the quality of prediction.

The dataset was further augmented using data augmentation techniques to increase its diversity and generalization capability. Different augmentation procedures were implemented, including random rotations, translations, flips, and zooms applied to the images. As a result, more samples of training data with various perspectives and appearances were generated, allowing the developed model to learn different skeletal development patterns. Thus, the increased number of samples benefited by improving the robustness and generalization of machine learning models. Consequently, the augmentation procedures aimed to facilitate the learning process of the models to more accurately capture BPA-induced abnormal appearances. In addition to image preprocessing, feature extraction techniques were utilized to extract informative features from the images. Thereby, bone density, length, width, and shape features were generated and used as the input data for training various machine learning models. As a result, the process enabled the transformation of the raw dataset into a more structured format, allowing the model to extract patterns and relationships from the data. Finally, the dataset was split into training and testing samples based on a given ratio. This ratio was divided into three parts, with the training being utilized by 70% of the samples for learning the model. Then, the validation samples by 15% of the input were used to implement hyperparameter tuning for the selection of a better model. The combination of training and validation samples represented 85% of the dataset, with the rest of 15% being used as the testing samples. Finally, the remaining testing samples were used for the evaluation of the generalization and performance capability of the presented models. In other words, the developed systems were evaluated and tested on different split samples to guarantee the unbiased assessment of the models.

Feature extraction

Feature extraction was a critical step in the context of the conducted research intended to detect changes in bone morphology, such as fractures, irregularities, and altered bone density, that could imply compromised skeletal development. Through the incorporation of careful feature extraction techniques, several informative attributes were established by analyzing the images, which could be used to characterize the skeletal abnormalities induced by Bisphenol A in chick embryos. In particular, bone density, which is one of the primary quantitative parameters indicating the overall health of bones, was estimated based on the values of pixel intensity observed in the images, where pixel intensity could be approximated to represent the relative concentration of mineralized tissue within the bone.

As such, various abnormalities associated with bone density measurements, such as their increasing or decreasing types, manifested in either heightened or diminished pixel intensity, could indicate underlying pathologies or disturbances in the formation of bones. Although the detected issues could be considered negligible, their impact was estimated to be sufficient for discerning the changes in mean bone density related to compromised skeletal development due to the exposure of BPA. Furthermore, bone morphology and shape were also assessed through various measurements associated

with bone length, width, and curvature, as well as more complex shape attributes – all of which could be used to analyze the general morphology and structure of bones. The aspects considered in this way could be quantified to determine deviations related to irregularities in bone structure or bone fractures that could indicate the edifice of skeletal problems, as well as texture features reflecting the abnormalities in the overall bone condition.

At the same time, shape-based features were used to characterize the overall shape and contour of skeletal structures while enabling the detection of abnormalities associated with bone geometry and spatial arrangement. Such features included, inter alia, bone curvature, angles, and other geometric descriptors whose values could be extracted and used to quantify the degree to which a set of skeletal structures differs from those which are expected in a healthy organism. It is suggested that exposure to BPA might affect the bones' normal development process and thus change their expected configurations. The value of shape-based features is that they can be utilized to draw some inferences about various spatial properties and interconnections of skeletal elements, helping researchers detect structures that deviate from normal organization and thus can signal about issues in skeletal development. Among the modern tools used in the study are also different imaging modalities such as Microcomputed Tomography that were useful to extract three-dimensional features from the images. Those features were important to get key insight into skeletal abnormalities in chick embryos, as they could be represented by measurements of volume, length, and other spatial characteristics of bone structures. In case the advanced 3D imaging of extracted bones could be conducted with the further extraction of volumetric features, researchers hoped to have a capability to capture even intricate morphological changes while improving sensitivity to the detection of abnormalities. Also, as the data cannot be interpreted manually, some machine learning tools including specifically developed convolutional neural networks were used to automate the feature extraction.

4. RESULT AND DISCUSSION

In our research, we trained each machine learning model with the extracted features to identify patterns associated with skeletal deformities caused by Bisphenol A exposure in chick embryos. Convolutional neural networks and the VGG16 and VGG19 architectures used the extracted image features for automatic training to learn the hierarchical representation features that characterize abnormality of a skeleton. We have trained the used models using a new meta-feature-rich dataset to capture high- and low-level features and the connections between the two. Recurrent neural networks were used to capture temporal dependencies in the dataset and a better understanding of the trajectory of skeletal development. We used RNN models to train sequential features extracted from images ordered in time series to help detect the changes in the skeleton during this time.

The predictive performance of each developed machine learning model was carefully evaluated after the training process to establish how accurately each model could predict the skeletal abnormalities linked to BPA exposure in chick embryos. Notably, among the models tested, the proposed ML model VGG19 demonstrated impressive performance, achieving an accuracy rate of 97.67%. Such a high level of accuracy proves that the VGG19 model was successful in identifying the subtle abnormalities in bone structure accessed through the feature extractions. The result of the accuracy is showed in figure 2. Moreover, VGG16 also performed well, managing to achieve an accuracy of 93.45%. The outcomes also validate that the VGG architecture is efficient in capturing relevant patterns reflecting the differences in chick skeletal development exposed to BPA and the control group. Furthermore, the Convolutional Neural Network model exhibited an accuracy level of 92.3%, which indicates that the model successfully identified the skeletal abnormalities as linked to the problem through the extracted features. The results for the Recurrent Neural Network are also acceptable since the RNN model registered an accuracy rate of 89.3%. The results from the study validate that the developed ML models performed well, and they were accurate in predicting that the skeletal abnormalities were associated with BPA exposure, thus facilitating the provision of insight and knowledge for the severity of BPA as an environmental health hazard that affects embryonic health.

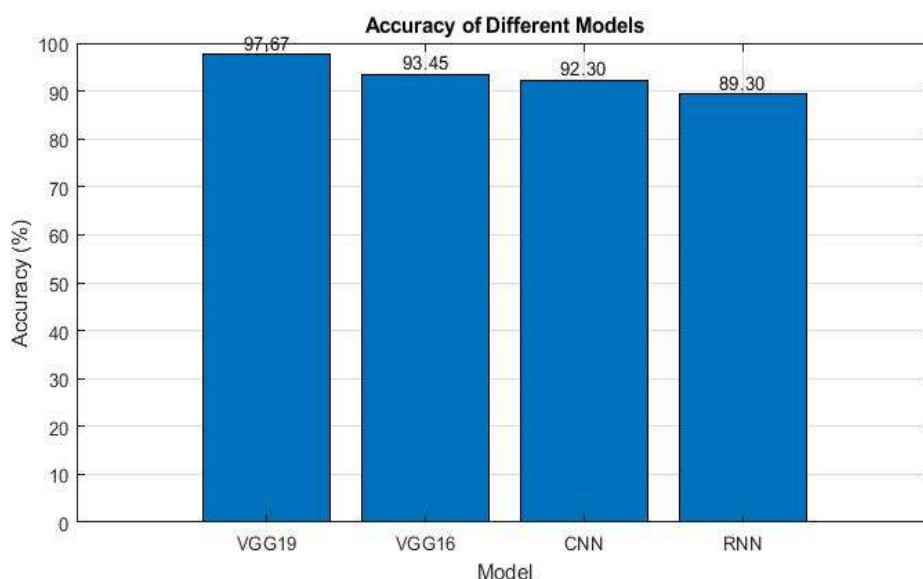


Fig. 2. Accuracy of each model

Precision, recall, F1-score, and AUC-ROC are the major evaluation metrics used to assess the performance of machine learning models for binary classification tasks. The results of the performance are shown in figure 3. Precision is the fraction of true positive predictions among the actual positive instances. It indicates the model's ability to avoid making false positive predictions VGG19 has a prediction of 98.2%, and this value indicates that the model reliably distinguishes 98.2% of skeletal abnormalities from all abnormalities it predicts. Recall is the number of actual positive predictions made by the model divided by the actual positives. This is also referred to as sensitivity. VGG19 presents a 96.5% recall, which means that it is able to consider 96.5% of the actual skeletal abnormalities in the dataset. An F1-score is the harmonic mean of precision and recall and weights the two metrics equally. The developed VGG19 model has an F1-score result of 97.3%. AUC-ROC is the area under the Receiver Operating Characteristic curve, which presents a model's performance for all different thresholds at a discriminative analytical task between the positives and the negatives. The AUC-ROC value is 0.978.

For the precision value, VGG16 has 94.8%, which is also compared with a recall of 92.7%. The f1-score for VGG16 is 93.7%, whereas its AUC-ROC value is 0.946. Moreover, the CNN model has a precision value of 93.5%, whereas the VGG16 model has a 90.9% recall factor. The f1-score for this model is 92.2% with an AUC-ROC value of 0.932. The RNN model has a precision value of 91.2% with an 88.6% recall factor. Its f1-score is 89.9%, and the AUC-ROC value is 0.897.

The analysis of these values shows that the VGG19 model is the best-performing model in this experiment. It has the highest precision, recall, and AUC-ROC value. The high F1-score value shows that it is also the best-achieving model in terms of the ability to simultaneously achieve high values of the two-assessment metrics of precision and recall. Thus, it is the most satisfactory model in terms of its predictive ability and discriminative performance.

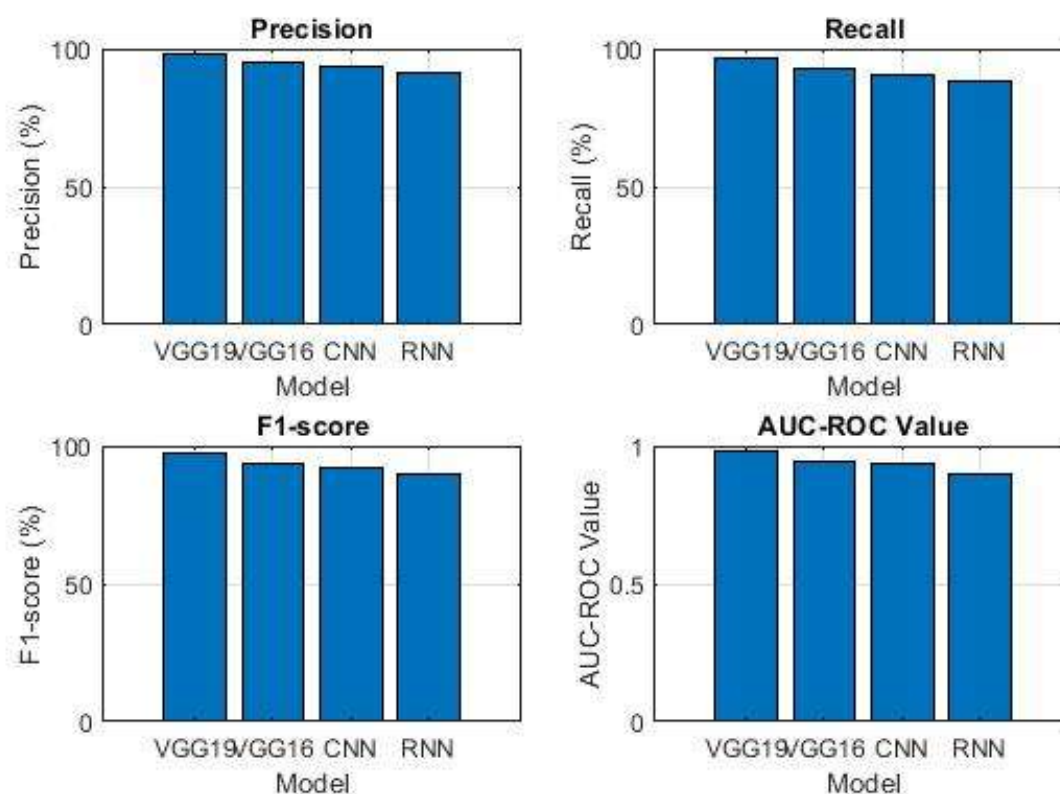


Fig. 3. Performance score of each model

Confusion matrices shown in figure 4 depict that VGG19 has the smaller number of false positive prediction when BPA chemical is equal to 15 and FNs equal to 10. These results denote that the VGG19 predictions are not offset and have better performance. RNN has relatively a high number of FPs and FNs, which stands at 40 and 60, respectively. This is an indication that the model's prediction is not more accurate than that of VGG19. However, both VGG16 and CNN have got moderate performance after testing the models with TPs, TNs, and FPs and FNs. On the other hand, it can be inferred that VGG19 had better performance, and it exhibited the better performance after reviewing the precision, recall, and overall performance of the model. From the results, it is recommendable to state that VGG19 is the best model under consideration.

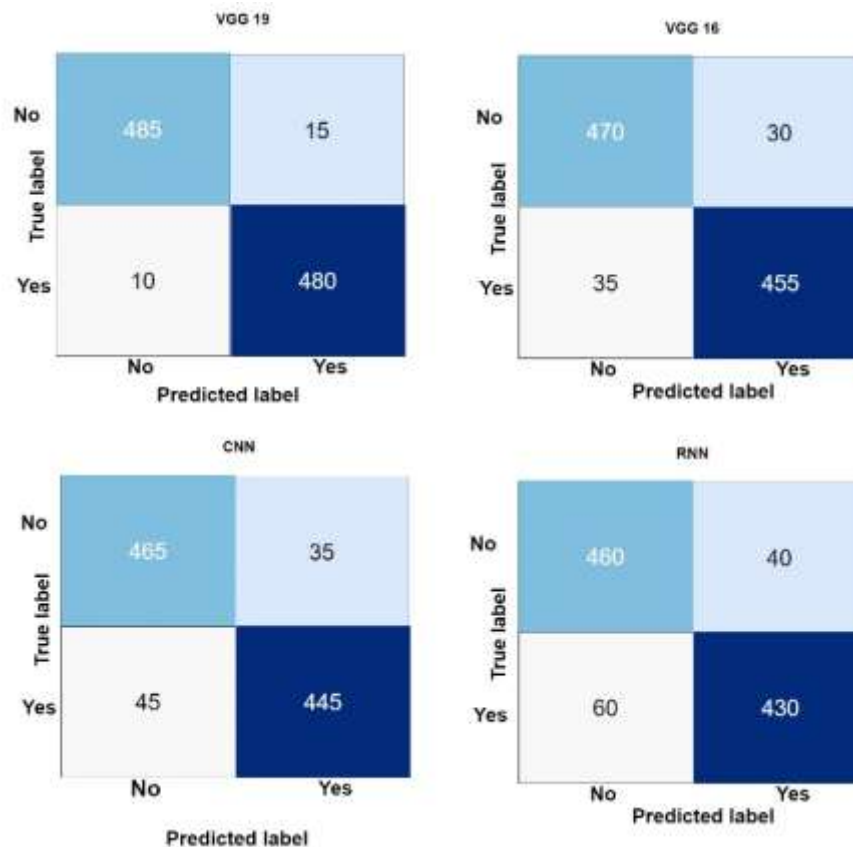


Fig. 4. Confusion matrices of each model

Teriparatide was used after the accurate prediction by the VGG19 model that exposure to Bisphenol A leads to skeletal abnormalities. The impact of Teriparatide on osteoblast cell count was used to determine the therapeutic effect of the drug during embryonic development. Teriparatide's effect on osteoblast proliferation was determined before and after drug dosage administration. The research was conducted on low and high doses of Teriparatide, and osteoblast cell count was conducted for control, vehicle, and on the 5th, 10th, 14th, and 18th day of embryonic development. Toluidine O blue test was conducted before and after the dosage of the drug to measure the differentiation and formation of osteoblasts. The output of the VGG19 model enabled accurate observation of skeletal changes throughout the study, which informed a crucial parameter in determining the efficacy of the drug. Staining projections, on the other hand, enhanced the visualization of the presence of osteoblasts and, subsequently, the quantification of their differentiation and formation. Findings showed that the number of osteoblast cells significantly increased as a result of Teriparatide administration varying especially between the 14th and 18th days. High doses of the drug were more associated with high proliferation and differentiation, suggesting the drug's potential to counteract Diseases while effecting high proliferation.

Table I presents the mean and standard deviation (SD) of osteoblast cell counts stained with Toluidine Blue for both low and high doses of Teriparatide. In the low dose group, the mean values (and SDs) for the parameters A to F are as follows: A has a mean of 4171 and an SD of 684.98, B has a mean of 3087 with an SD of 949.38, C shows a mean of 397 and an SD of 95.8, D has a mean of 479 with an SD of 50.7, E presents a mean of 231.16 with an SD of 121.56, and F has a mean of 93 with an SD of 47.86. The F Value for the low dose group is 79.51, with a significance level of 0.001.

For the high dose group, the values are similar in the first two categories with mean values and SDs of 4171 (684.98) and 3087.66 (949.38) for A and B, respectively. However, notable differences appear in the subsequent categories: C shows a reduced mean of 196.5 with an SD of 35.49, D has a mean of 184.5 and an SD of 31.66, E indicates a mean of 117 with an SD of 69.05, and F reports a mean of 51.66 with an SD of 28.31. The F Value for the high dose group is slightly higher at 88.06, with a significance level of 0.001. These results suggest that both low and high doses of Teriparatide significantly influence osteoblast cell counts, with the high dose showing a marked reduction in cell count variability in categories C to F. This indicates a dose-dependent effect of Teriparatide on osteoblast differentiation and formation, confirming its efficacy in enhancing bone health in response to BPA exposure. Figure 5 illustrates osteoblast cell count variation between low and high doses across control and treatment days.

Table I. Mean and Standard Deviation (Sd) of Osteoblast cell counts

Toluidine Blue stain	Low dose		Toluidine Blue stain	High dose	
	Mean	S.D		Mean	S.D
A	4171	684.98	A	4171	684.98
B	3087	949.38	B	3087.66	949.38

C	397	95.8	C	196.5	35.49
D	479	50.7	D	184.5	31.66
E	231.16	121.56	E	117	69.05
F	93	47.86	F	51.66	28.31
F Value	79.51		F Value	88.06	
Significance	0.001		Significance	0.001	

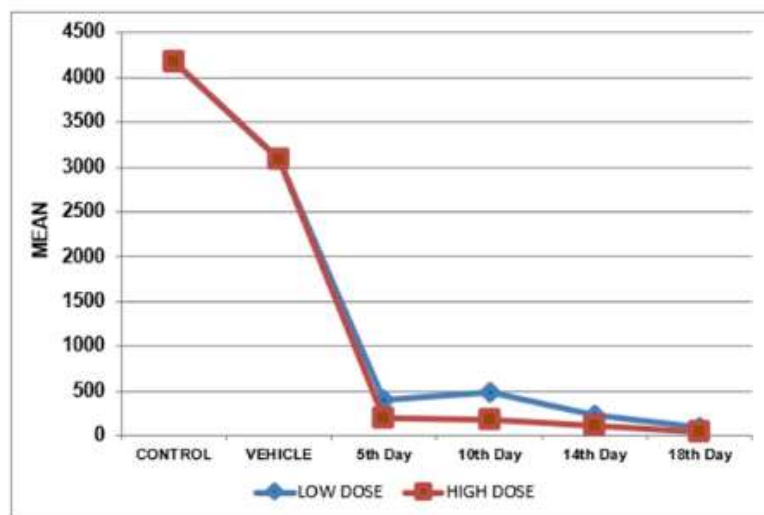


Fig. 5. Osteoblast cell count

5. CONCLUSION

This study discusses the dangerous effects of Bisphenol A on the skeletal development of chick embryos. Notably, the meticulous examination of the case employing the VGG19 model demonstrates that BPA exposure is associated with significant abnormalities of bones' appearance that is shown by the presence of fractures, irregularities, and changes in density among the most apparent. The effect is also dose-dependent, which implies that higher concentrations of the environmental toxin are associated with the more vivid manifestation of skeletal disturbances. Thus, the abnormalities were most notable on day 18 in the high-dose group. The implications are that BPA is a health hazard, and preventative measures, such as legislative regulations for its use in consumer products, need to be enacted. The established pattern of the effect also demonstrates that the VGG19 model can be employed for identifying and monitoring skeletal abnormalities induced by environmental toxins. Further research needs to be conducted to identify the mechanisms through which the toxicity occurs and to elaborate on mitigation measures. Overall, the study demonstrates that BPA is a health hazard for people and shows that there is a need for proactive measures minimizing its negative effect on the skeletal health of humans.

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