An analysis of Heptoprotective effects of plant extract on albino rats by Deep Learning analysis using ResNet and VGG16 algorithms

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Abstract: The hepatoprotective and anti-inflammatory effects of Terminalia catappa L. and Pergularia daemia hydroalcoholic extracts in albino rats with CCl4-induced liver damage (HAETC and HAEPD, respectively). The extracts significantly reduced the harm induced by CCl4 when given orally at a dosage of 200mg/kg. They also showed strong hepatoprotective and antioxidant properties. Wistar albino rats in good health were divided into seven groups, each with six animals. Group I acted as the control and received no treatment, whereas Group II was given 30% CCl4 (1ml/kg, intraperitoneally) to cause hepatotoxicity. After administering Silymarin (25 mg/kg, orally) as per protocol, Group III underwent CCl4 therapy. The CCl4-induced rats showed significant reductions in antioxidant enzymes like SOD, catalase, glutathione peroxidase (GPX), and glutathione-S-transferase (GST), as well as increases in serum marker enzymes like SGOT, SGPT, alkaline phosphatase (ALK), ACP, LDH, and lipid peroxidation (LPO) activity. However, rats given HAEPD and HAETC showed significant improvements, returning these parameters to levels that are almost normal (P 0.001).The hepatoprotective and antioxidant benefits of Terminalia catappa L. (HAETC) and Pergularia daemia (HAEPD) against CCl4-induced hepatotoxicity in rats were further validated by histopathological analysis of liver tissues. It encourages the use of pre-trained CNN architectures, such as VGG16 and ResNet (Residual Network), in the proposed method, which uses convolutional neural networks (CNNs) for histopathological image analysis to assess the hepatoprotective properties of Pergularia daemia and Terminalia catappa L. leaf extracts against CCl4-induced hepatotoxicity. These well-known CNN models may be modified for precise and effective histopathological image interpretation, assisting in the evaluation of the impact of plant extracts on liver health.

Index Terms- Pergularia daemia, Terminalia catappa, Hepatoprotective, Antioxidant, Histopathology, CCl4, CNN, VGG16, ResNet, CNN, Deep learning..

1. Introduction

In the metabolism, detoxification, and excretion of several xenobiotic substances, the liver is essential. The liver is commonly a target for hazardous compounds because of its anatomical position and substantial potential for xenobiotic metabolism. Hepatic damage can also be caused by xenobiotics, excessive pharmacological therapy, exposure to environmental toxins, and persistent alcohol consumption. Viral infections stand out as the main cause of liver damage. Notably, it is known that cancer and chemotherapy treatments can cause liver toxicity, which is characterized by the production of free radicals and reactive oxygen species, which are important in the pathophysiology of the liver.

Using a rodent model of liver injury brought on by carbon tetrachloride (CCl4), a chemical hepatotoxin that causes free radical-mediated hepatocellular damage, is a well-established in-vivo method for researching hepatoprotective substances. It is known that CCl4 can cause hepatotoxicity in two stages. In the early stage, Cytochrome P450 converts CCl4 into trichloromethyl radicals, which causes membrane lipid peroxidation and ultimately results in cell necrosis. Kupffer cell activation and the release of pro-inflammatory mediators are both part of the second phase.

Traditional medical procedures have resorted to plant-based treatments in search of secure and efficient hepatoprotective substances. Herbal medications have become more popular as a result of their efficacy, affordability, and safety. These plants include Terminalia catappa L. and Pergularia daemia, both of which have long been valued for their therapeutic qualities. In folk and Ayurvedic medicine, Pergularia daemia, a foetid-smelling laticiferous twiner found in the plains of hot regions of India, has been used for a variety of treatments, including hepatoprotective effects, wound healing, antidiabetic effects, cardiovascular advantages, and antifertility.

The significant, spreading tree Terminalia catappa, often known as Indian almond and a member of the Combretaceae family, is found across tropical areas. According to reports, this plant's leaves contain aphrodisiac, anti-clastogenic, anti-cancer, anti-HIV, hepatoprotective, anti-inflammatory, anti-hepatitis, and anti-diabetic properties.

The goal of the present research is to improve our understanding of the hepatoprotective properties of hydro-alcohol extracts from the leaves of Terminalia catappa L. and Pergularia daemia L. against CCl4-induced rat liver damage. To evaluate the effect of these plant extracts on liver health, the study introduces the use of convolutional neural networks (CNNs) for histopathological image interpretation. This technique is predicted to offer insightful information on the microscopic alterations taking place in liver tissues, assisting in a thorough assessment of the hepatoprotective effects of the tested extracts.

2. Materials And Methods

2.1 Gathering a sample of plants

From South Poigainallur Village in the Nagapattinam district of Tamilnadu, India, fully grown leaves of Pergularia daemia and Terminalia catappa (Red leaves) were harvested in August and September 2012. Dr. P. Jayaraman, of the Plant Anatomy Research Centre in West Tambaram, Chennai, classified the plants according to taxonomy classification. Pergularia daemia (PARC/2013/2118) and Terminalia catappa (PARC/2014/2063) voucher specimens were acquired.

2.1 Extract Preparation

The mature leaves were mechanically ground into a powder, shade-dried at room temperature, then extracted with alcohol. It was extracted using a Soxhlet device at 65 °C till discolouration. After full evaporation, the resultant extract was put into sealed containers and kept in the fridge until it was needed.

2.3 Experimental Animals & Design

The King Institute in Guindy, Chennai, Tamil Nadu, India provided healthy Wistar strain male rats (2-3 months old, weighing 150-200g). From Saveetha University in Chennai, ethical approval (No: SU/BRULAC/RD/012/2014), was acquired. The rats were given a five-day acclimatization period, standard housing, a standard pelleted meal, and unlimited access to water.

The animals were divided into seven groups (each with 6 rats) as follows:

1. **Control (Group I):** Olive oil (vehicle) (1 ml/kg b.wt, p.o.) every 72 hrs for 10 days.
2. **CCl4 Intoxicated (Group II):** 30% CCl4 (1 ml/Kg, b.wt, i.p.) every 72 hrs for 10 days.
3. **Standard (Group III):** Silymarin (25mg/kg, b.wt, p.o.) for 15 days and 30% CCl4 (1ml/Kg, b.wt, i.p.) every 72 hrs for 10 days.
4. **Pre-treatment (Group IV):** Hydroalcohol extract of Pergularia daemia (200 mg/kg, b.wt, p.o.) for first 15 days, followed by 30% CCl4 (1ml/Kg, b.wt, i.p.) every 72 hrs for next 10 days.
5. **Pre-treatment (Group V):** Hydroalcohol extract of Terminalia catappa (200 mg/kg, b.wt, p.o.) for first 15 days, followed by 30% CCl4 (1ml/Kg, b.wt, i.p.) every 72 hrs for next 10 days.
6. **Post-treatment (Group VI):** 30% CCl4 (1ml/Kg, b.wt, i.p.) every 72 hrs for first 10 days, followed by hydroalcohol extract of Pergularia daemia (200 mg/kg, b.wt, p.o.) for next 15 days.
7. **Post-treatment (Group VII):** 30% CCl4 (1ml/Kg, b.wt, i.p.) every 72 hrs for first 10 days, followed by hydroalcohol extract of Terminalia catappa (200 mg/kg, b.wt, p.o.) for next 15 days.

2.4 Blood Collection

Animals were decapitated in the cervical region as a kind of sacrifice on the 25th day. For biochemical analysis, serum was isolated after blood was drawn, allowed to clot. Evaluation of Liver Function -SGOT, SGPT, ALK, LDH, and ACP were among the biochemical markers that were measured using conventional techniques.

2.5 Evaluation of the Properties of Antioxidants

In order to assess the levels of lipid peroxides, catalase, glutathione peroxidase, and superoxide dismutase in liver tissue. Glutathione-S-transferase (GST) and total reduced glutathione (GSH) levels were also assessed.

Histopathological Studies - For microscopic inspection, liver tissues were fixed, prepared, sectioned, and stained with haematoxylin and eosin.

2.6 CNNs (VGG16 and ResNet) for Histopathological Image Analysis

CNNs (VGG16 and ResNet) that have already been trained to analyze histopathology images were used. The CNNs were used to detect and evaluate microscopic characteristics suggestive of hepatoprotective effects from the digitized liver tissue slices.

2.7 Statistical Analysis

SPSS 20 and GraphPad Prism 6 were used to conduct the statistical analysis. Six samples from each group were used to calculate the mean and standard deviation of the results. A one-way analysis of variance (ANOVA) was employed, taking differences seriously at p 0.05 and employing a post hoc multiple comparison test.

3. CNN Models Of Deep Learning

Pre-trained CNN architectures like VGG16 (Visual Geometry Group 16) and ResNet (Residual Network) is related to their application in histopathological image analysis for assessing the hepatoprotective properties of Pergularia daemia and Terminalia catappa L. leaf extracts against CCl_4-induced hepatotoxicity in rats.

3.1 VGG16 (Visual Geometry Group 16)

VGG16 is a convolutional neural network architecture that was proposed by the Visual Geometry Group at Oxford. It is characterized by its simplicity and uniform architecture, consisting of 16 weight layers (13 convolutional layers and 3 fully connected layers). Each convolutional layer has a small receptive field of 3x3 and is followed by a ReLU (Rectified Linear Unit) activation function. VGG16 is known for its ability to capture complex patterns in images and is widely used in image classification tasks.

3.2 ResNet (Residual Network)

ResNet is a deep neural network architecture that introduced the concept of residual learning. It addresses the problem of vanishing gradients in very deep networks by using skip connections or shortcuts to jump over some layers. ResNet has residual blocks, and the key idea is to learn residual functions with reference to the layer inputs, allowing the model to learn the identity mapping easily. This enables the training of very deep networks, leading to improved performance in various computer vision tasks.
Histopathological image analysis for assessing hepatoprotective properties:

1. **Feature Extraction:** Both VGG16 and ResNet act as feature extractors. They take histopathological images as input and automatically learn hierarchical features at different levels of abstraction.
2. **Transfer Learning:** Pre-trained models like VGG16 and ResNet have been trained on large datasets (e.g., ImageNet) for image classification tasks. In the proposed method, these pre-trained models can be used as a starting point, and their weights can be fine-tuned on the specific histopathological images related to hepatotoxicity assessment.

3. **Histopathological Image Interpretation:** Features from the pre-trained models are then used to interpret histopathological images. These features capture important patterns and structures in the images, allowing the model to distinguish between healthy and damaged liver tissues.
4. **Model Modification:** The architecture of pre-trained models might be modified or extended based on the specific requirements of the histopathological image analysis task.
5. **Prediction and Evaluation:** The modified model is then used to predict the hepatotoxicity levels in the given histopathological images. The performance of the model is evaluated based on metrics such as accuracy, precision, recall.

4. Proposed Methods:

Step 1: Data Collection: Obtain histopathological images of liver samples from rats in different experimental groups, including those treated with Pergularia daemia and Terminalia catappa L. extracts.

Step 2: Pre-processing: Prepare the images by resizing, normalizing, and potentially augmenting them to ensure consistency and quality for CNN analysis.

Step 3 : CNN Selection: Choose appropriate pre-trained CNN architectures, such as VGG16 or ResNet, which have been proven effective for image analysis tasks.

Step 4: Transfer Learning: The pre-trained CNN models on the collected histopathological images.

Step 5: Feature Extraction: The trained CNN to extract meaningful features from the histopathological images that can indicate the extent of liver damage and recovery.

Step 6: Classification/Regression: Develop a classification or regression model using the extracted features to assess the degree of hepatoprotection offered by Pergularia daemia and Terminalia catappa L. extracts.

Step 7: Statistical Analysis: Apply appropriate statistical tests to evaluate the significance of the results obtained through CNN-based image analysis.

In order to objectively evaluate and quantify the hepatoprotective benefits of the plant extracts, the proposed approach makes use of computer vision and deep learning techniques, enabling a more in-depth and fact-based knowledge of their influence on liver health.

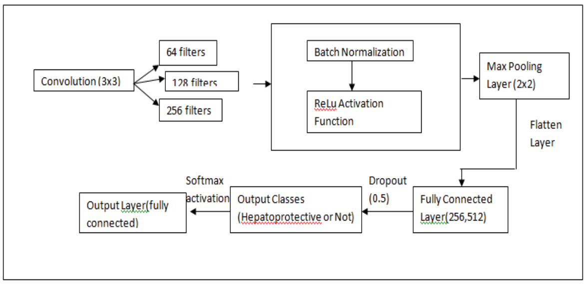

Fig 1: CNN Architecture for Hepatoprotective Effects Evaluation

This architecture is a simplified representation. The resolution of the images and the size of the dataset will influence the final layer, which has two nodes representing the classes 'hepatoprotective' and 'Not hepatoprotective.' In the process, a pre-trained CNN like VGG16 or ResNet is utilized for feature extraction. The subsequent step involves appropriate preprocessing of histopathological images, including resizing, normalization, and augmentation, modified to the dataset and problem features. Additionally, transfer learning is used to take advantage of the knowledge earned through pre-training on a sizable dataset for a comparable job.

5. Results & Discussion

When compared to the control group, the CCl4-treated group showed a substantial (p 0.001) rise in hepatospecific blood indicators such SGOT, SGPT, ALP, ACP, and LDH. However, the activity of these enzymes were seen to normalize after pre- and post-treatment with HAEPD, HAETC, and silymarin. In order to improve the assessment procedure, deep learning CNN models VGG16 and ResNet were also included in this study. Based on the distinctive properties of the histopathology pictures, the architectural specifics and hyperparameters of these models were meticulously modified. Transfer learning techniques were used to enhance the models' performance while assessing the hepatoprotective effects of compounds by utilizing the information obtained from pre-training on big datasets.

This research's combination of VGG16 and ResNet enables a more thorough examination of histopathological pictures, improving our comprehension of how HAEPD and HAETC affect liver health. An in-depth analysis of the experimental results is made possible by the use of these cutting-edge models.

6. Conclusion

The analysis of the hepatoprotective properties of leaf extracts from Terminalia catappa L. (HAETC) and Pergularia daemia (HAEPD) against CCl4-induced liver damage in rats has produced important findings. For a full analysis of histopathology pictures, the study used a comprehensive methodology that combined conventional biochemical studies with cutting-edge deep learning CNN models, VGG16 and ResNet.

This research not only offers insightful information on the hepatoprotective abilities of HAEPD and HAETC, but it also emphasizes the effectiveness of cutting-edge computational approaches in pharmaceutical research. Future research might broaden the use of deep learning models for a more thorough investigation of herbal therapies in hepatotoxicity and examine the molecular processes behind the reported effects.

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