AN EXPERIMENTAL STUDY OF CHELIDONIUM 30 , 200 AND CROTALUS HORRIDUS 30 , 200 IN TREATING PHENYLHYDRAZINE INDUCED HYPERBILIRUBINEMIA IN WISTAR ALBINO RATS

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Abstract : Hyperbilirubinemia, sometimes known as jaundice, most commonly affects infants, though it can also affect adults and children. Even while infants' bilirubin levels are inherently higher, the liver often efficiently removes the excess. The outcome is hyperbilirubinemia, which typically disappears in a few weeks. In severe cases, it could lead to kernicterus. Early identification and treatment are essential to prevent serious complications and long-term effects. The efficiency of early hyperbilirubinemia identification and therapy has increased because to medical advancements. Despite the effectiveness of conventional treatments like exchange transfusion and phototherapy, alternative therapies are growing in popularity. Homeopathy uses very diluted natural remedies to provide a complete approach to treatment. I plan to examine the efficacy of Chelidonium majus and Crotalus horridus in lowering hyperbilirubinemia in albino rats with phenylhydrazine-induced hyperbilirubinemia. The objective is to determine whether the homoeopathic drugs Chelidonium majus 30, 200 and Crotalus horridus 30, 200 have an antihyperbilirubinemic effect in phenylhydrazine-induced albino rats in order to compare the effects of phenobarbital and homoeopathic therapy. Standard humidity and temperature levels, as well as a 12-hour light-dark cycle, must be maintained during the study.Before the study starts, rats must be given a regular diet and drink for a week so they can get used to the lab environment.All rats must be weighed and have blood drawn prior to induction in order to detect conjugated and unconjugated bilirubinemia. In rat models, phenylhydrazine causes jaundice, which leads to hyperbilirubinemia.In addition to a group that received standard phenobarbital treatment, the recommended dosage for Chelidonium majus 30,200 and Crotalus horridus 30,200 is two pills given orally with ten drops of filtered water every three hours.Blood samples from rats are needed to test conjugated and unconjugated bilirubin and assess the presence of jaundice. Six groups of three animals each were created. The study of blood bilirubin levels—conjugated, unconjugated, and total—determines the outcome. It can be shown that the homoeopathic treatments Crotalus horridus and Chelidonium majus have anti-hyperbilirubinemic benefits despite being a comparative study.

Index Terms **- Hyperbilirubinemia, Chelidonium majus , Crotalus horridus ,Homoeopathy**

1. INTRODUCTION

The yellowing of body tissue caused by an accumulation of increased bilirubin is known as hyperbilirubinemia. Poor elimination or increased bilirubin production are the most common causes. Serum bilirubin levels are less than 1 mg/dl[1]. Bilirubin is the breakdown product of hemoglobin and other hemoproteins. Because of intrinsic hydrogen bonds, bilirubin is insoluble in water, and the liver must use enzyme-mediated glucuronidation to eliminate it. The majority of blood bilirubin is normally unconjugated and firmly attached to circulatory albumin [2]. Bilirubin is divided into two halves: conjugated (direct) and unconjugated (indirect). Elevated levels of these lead to jaundice [1] . Bilirubin is produced when heme is broken down. In the first phase, heme transforms into biliverdin; in the second phase, reductase converts biliverdin into unconjugated bilirubin. During the conjugation process, albumin transports unconjugated bilirubin to the liver because it is insoluble in water. Unconjugated bilirubin is subsequently converted to conjugated bilirubin by the liver. The intestines get conjugated bilirubin. Conjugated bilirubin is absorbed by intestinal cells and then released as urobilinogen in urine, while the remaining is eliminated as stercobilinogen in stool. Changes in these metabolisms lead to jaundice [3]. Bigger celandine is another name for Chelidonium majus. The family is called Papaveraceae. The primary constituents of the plant include flavonoids, phenolic acid, and isoquinoline alkaloids.

Chelidonium majus has a number of pharmacological effects, including hepatoprotective, anti-inflammatory, choleretic, immunomodulatory, analgesic, and antibacterial. It mitigates the hepatotoxic consequences[4]. Crotalus horridus, the rattle snake, is chemically composed of soda cyanhydrates. It helps treat a variety of illnesses. Jaundice, cholera, plague, yellow fever, and other hemorrhagic signs[5]. This study aims to show that after phenylhydrazine induction, Chelidonium majus 30, 200 and

Crotalus horridus 30, 200 exhibit anti-bilirubin activity in wistar albino rats. In this study, there are six groups of eighteen albino rats, each consisting of three rats. All of the animals will be induced with phenylhydrazine. Group 1 is a normal control group that does not take any medications. Group 2 includes Chelidonium majus 30. Group 3 includes Chelidonium majus 200. Group 4 contains Crotalus horridus 30, Group 5 contains Crotalus horridus 200, and Group 6 receives 60 mg/kg of phenobarbital daily.
Every three hours, homoeopathic drugs will be given in water doses of two pills in ten drops of distilled water. During the trial, bilirubin levels are assessed both prior to and following treatment.

2. NEED OF THE STUDY

Jaundice is a multifaceted illness[6] Clinical signs of jaundice appear when the bilirubin level exceeds 2 mg/dl[7].
Jaundice symptoms include anemia, sclera yellowing, dark yellow urine, yellow skin, fever, vomiting, pale stool, and widespread pruritis. It can result in mental illnesses including kernicterus, coma, or even death if treatment is not received[6]. The pathogenic aspect of jaundice has three stages: phases that take place prior to, during, and following the liver.
During the prehepatic phase, hemolysis and hematoma reabsorption result in an excess of bilirubin, usually unconjugated. Hepatocyte failure, poor bilirubin conjugation or excretion, metabolic or genetic condition, cholestatic liver disease, and viral or alcoholic hepatitis are all included in the intrahepatic phase. The posthepatic phase after conjugation involves bile flow obstruction due to gallstones, pancreatic or biliary tract malignancies, strictures, and pancreatitis [8]. Research has shown that Chelidonium majus , a homoeopathic remedy , lowers bilirubin levels [9].
I wish to show the anti-hyperbilirubinemic effects of Chelidonium majus 30, 200 and Crotalus horridus 30, 200 in albino rats given phenylhydrazine in the current era of experimental study. Additionally, I would like to contrast the effects of homoeopathic medication and phenobarbital.

3. RESEARCH METHODOLOGY

3.1 STUDY SETTING

The intention of this study was to assess the effectiveness of the homoeopathic remedies Crotalus horridus and Chelidonium majus in treating wistar albino rats hyperbilirubinemia caused by phenylhydrazine . For this experiment , eighteen wistar albino rats were collected . Every rat was divided into six groups , each of which had three rats .

3.2 SELECTION OF STUDY SAMPLES

For the study , healthy male Wistar albino rats weighing between 180 to 200 g at 7 to 8 weeks of age were recruited .

3.3 STUDY DESIGN

Rats with hyperbilirubinemia produced by phenylhydrazine were used in this experimental investigation .

3.4 ANIMAL ALLOTEMENT

GROUP 1 : Negative Control group , treated with phenylhydrazine (75mg/kg intraperitoneally not more than 1 ml) GROUP 2 :Treating with Chelidonium majus 30 , 2 pill in 1 dram bottle of distilled water 0.5ml, 2 times , orally GROUP 3: Treating with Chelidonium majus 200, 2 pill in 1 dram bottle of distilled water 0.5ml , 2 times , orally GROUP 4: Treating with Crotalus horridus 30, 2 pill in 1 dram bottle of distilled water 0.5ml , 2times , orally GROUP 5: Treating with Crotalus horridus 200 , 2 pill in 1 dram bottle of distilled water 0.5 ml , 2times , orally GROUP 6 : Treating with Phenobarbital is administered 60mg/kg daily 0.5ml , orally.

3.5 METHODOLOGY:

DAY 1 : Obtaining a blood sample (no more than 1 millimeter) from the left or right retroorbital vein . All the rats from each group will have blood drawn , and the direct , indirect and total serum bilirubin levels will be measured . Following blood collection , all 36 rats receive an intraperitoneal injection of 75 mg/kg body weight of phenylhydrazine (no more than 1 ml).

DAY 2 : All 36 rats received intraperitoneal injections of phenylhydrazine at a dose of 75 mg/kg body weight (no more than 1 ml)
DAY 3 : Taking a blood sample (no more than 1 ml) from the retroorbita vein and measuring the serum bilirubin level in each of the groups . One hour later , giving group II the homoeopathic medication Chelidonium majus 30 , 2 pill in 1 dram of distilled water 0.5 ml twice morning and evening orally , group III the homoeopathic medication Chelidonium majus 200 , 2 pill in 1 dram of distilled water 0.5 ml twice morning and evening orally , group IV the homoeopathic medication Crotalus horridus 30 , 2 pill in 1 dram of distilled water 0.5 ml twice morning and evening orally , group V the homoeopathic medication Crotalus horridus 200 , 2 pill in 1 dram of distilled water 0.5 ml twice morning and evening orally , group VI Phenobarbital 60 mg/Kg 0.5 ml orally .

DAY 3 - DAY 13 : Give homoepathic medicine Chelidonium 30 ,2 pill in 1 dram of distilled water 0.5 ml , 2 times morning and evening orally in group II , Chelidonium 200 , 2 pill in 1 dram of distilled water 0.5 ml , 2 times morning and evening orally for group III , Crotalus horridus 30 , 2 pill in 1 dram of distilled water 0.5 ml , 2 times morning and evening orally for group IV , Crotalus horridus 200 , 2 pill in 1 dram of distilled water 0.5 ml , 2 times morning and evening orally for group V and Phenobarbital 60mg/kg , 0.5ml orally for group VI.

DAY 14 : Obtaining a blood sample from the animal's retroorbital vein (no more than 1 ml) and killing it . Following sacrifice , the liver is taken out and put in a formalin buffer solution for histological analysis .

3.6 OUTCOME ASSESSMENT

To measure total bilirubin , direct bilirubin and indirect bilirubin , blood has been extracted from each animal . The effectiveness of homoeopathic medication was determined to be statistically significant by comparing each value between groups in order to understand changes in values . Chelidonium majus 30 , 200 and Crotalus horridus 30 , 200 were shown to be effective .

4. OBSERVATION AND RESULTS

The study evaluated the effectiveness of Chelidonium majus 30 , 200 and Crotalus horridus 30 , 200 in treating phenylhydrazine induced hyperbilirubinemia in animals . All groups first developed reduced activity , poor intake and exhaustion after phenylhydrazine administration . After treatment total , direct and indirect bilirubin levels were measured and statistically compared between groups to assess the remedies impact on bilirubin reduction.

Table 1 – Blood Parameters for Direct Bilirubin

	After inducing	Chelidonium majus 30	Chelidonium majus 200	Crotalus horridus 30	Crotalus horridus 200	Phenobarbital
RAT 1	5.04	0.05	0.13	0.07	0.13	0.15
RAT 2	5.08	0.08	0.19	0.07	0.11	0.15
RAT 3	5.06	0.06	0.14	0.06	0.13	0.12

Figure 1 – Graphical comparison of Direct bilirubin between all the groups

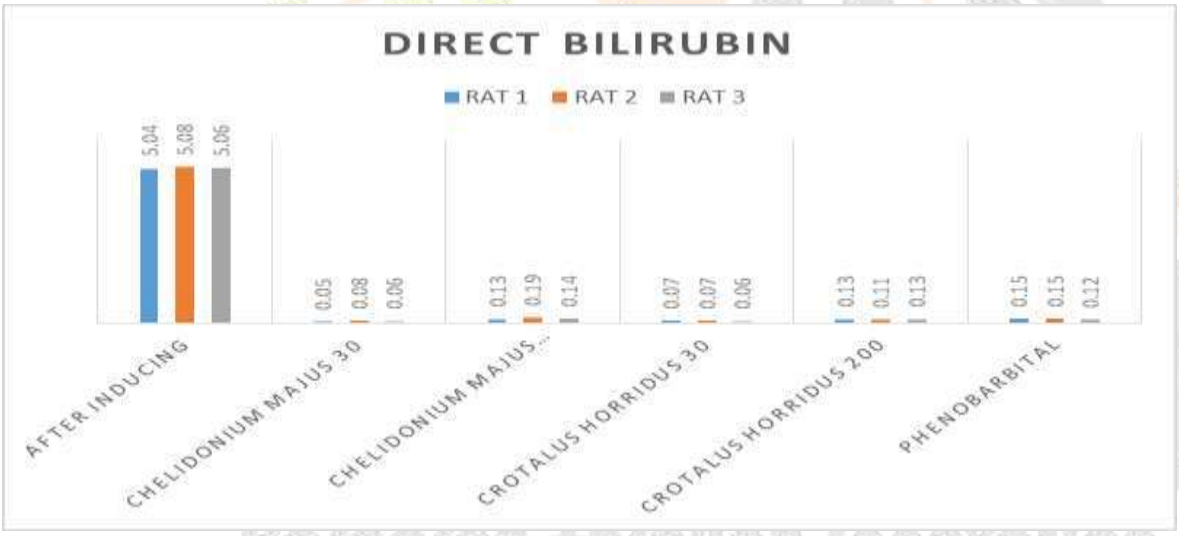


Table 2 – Blood Parameters for Indirect Bilirubin

	After inducing	Chelidonium majus 30	Chelidonium majus 200	Crotalus horridus 30	Crotalus horridus 200	Phenobarbital
RAT 1	5.45	0.19	0.3	0.03	0.2	0.54
RAT 2	5.78	0.16	0.2	0.02	0.26	0.49
RAT 3	5.32	0.17	0.3	0.03	0.3	0.54

Figure 2 – Graphical comparison of Indirect bilirubin between all the groups

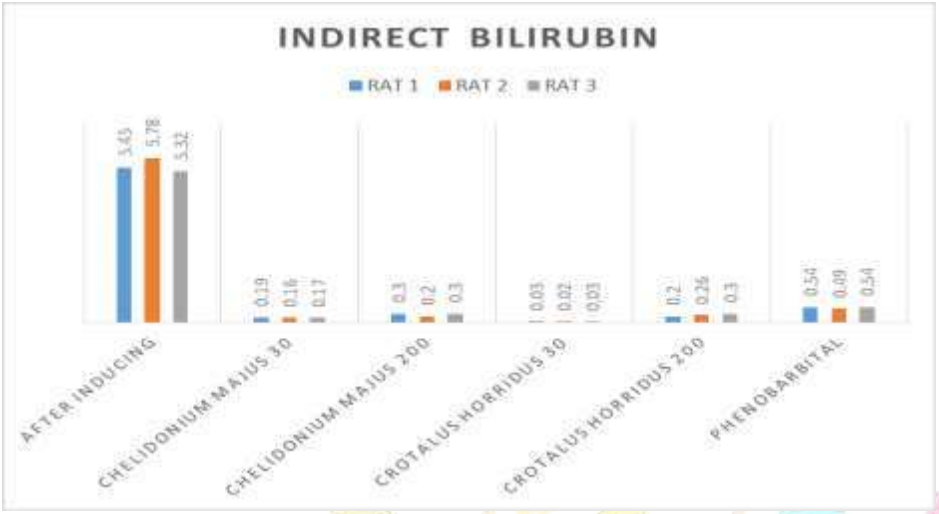
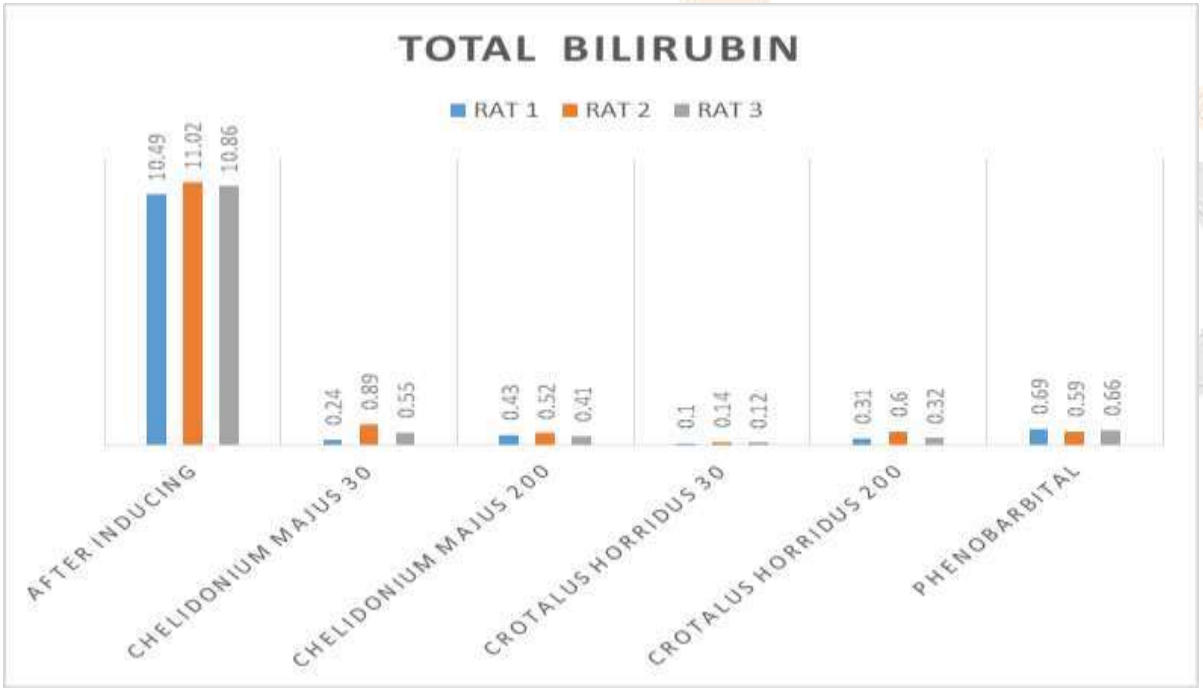


Table 3 – Blood Parameters for Total Bilirubin

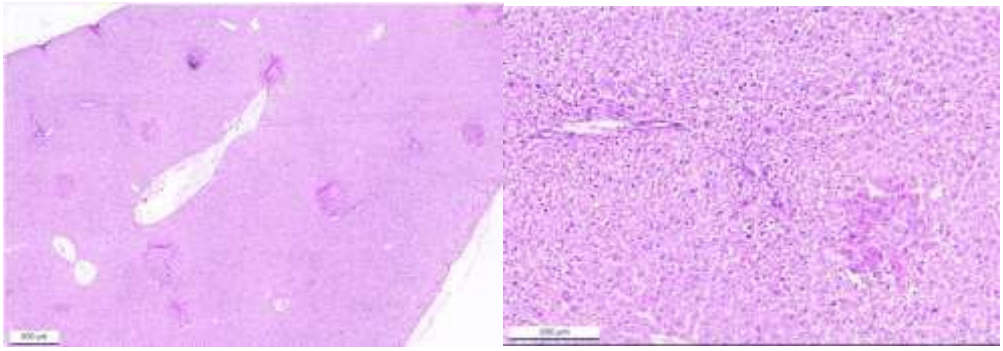
	After inducing	Chelidonium majus 30	Chelidonium majus 200	Crotalus horridus 30	Crotalus horridus 200	Phenobarbital
RAT 1	10.49	0.24	0.43	0.10	0.31	0.69
RAT 2	11.02	0.89	0.52	0.14	0.60	0.59
RAT 3	10.86	0.55	0.41	0.12	0.32	0.66

Figure 3– Graphical comparison of Total bilirubin between all the groups



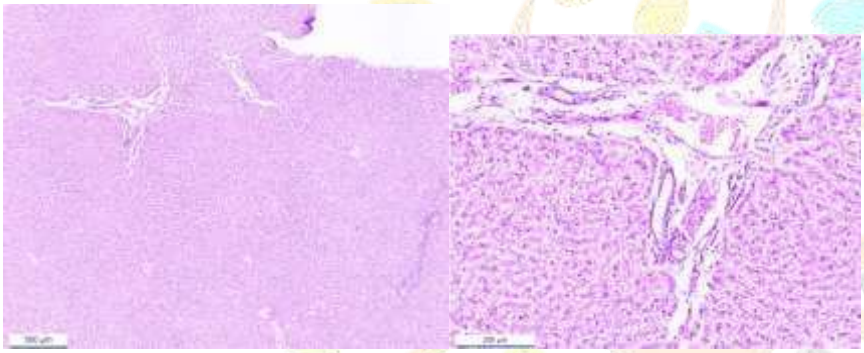
HISTOPATHOLOGICAL RESULT

Fig 4



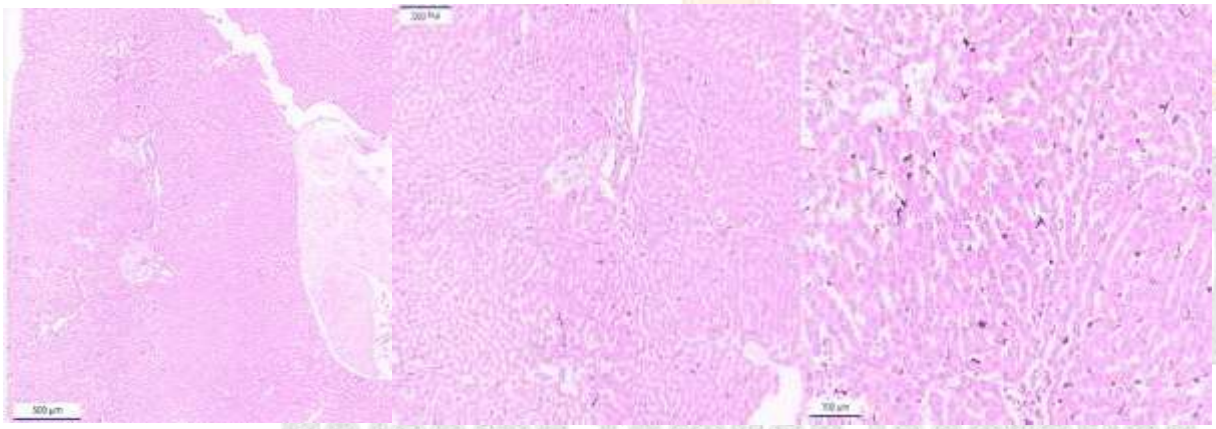
GROUP 1 : NEGATIVE CONTROL GROUP - Multifocal necrotic foci with mild peri – portal and peri – central fibrosis observed within the hepatic parenchyma .

Fig 5



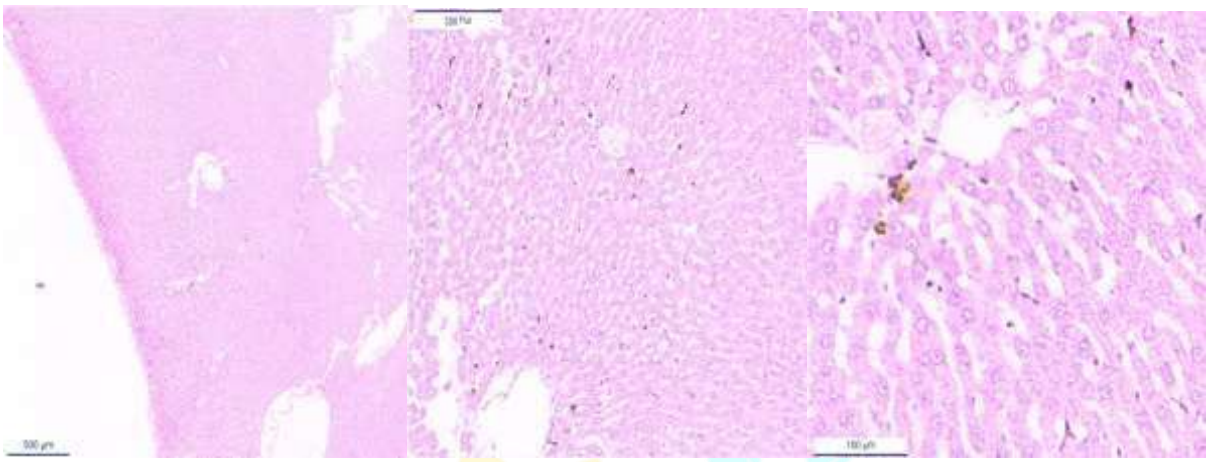
GROUP 2 – CHELIDONIUM MAJUS 30 - Multiple necrotic foci with mild peri – portal and peri – central fibrosis observed within the hepatic parenchyma .

Fig 6



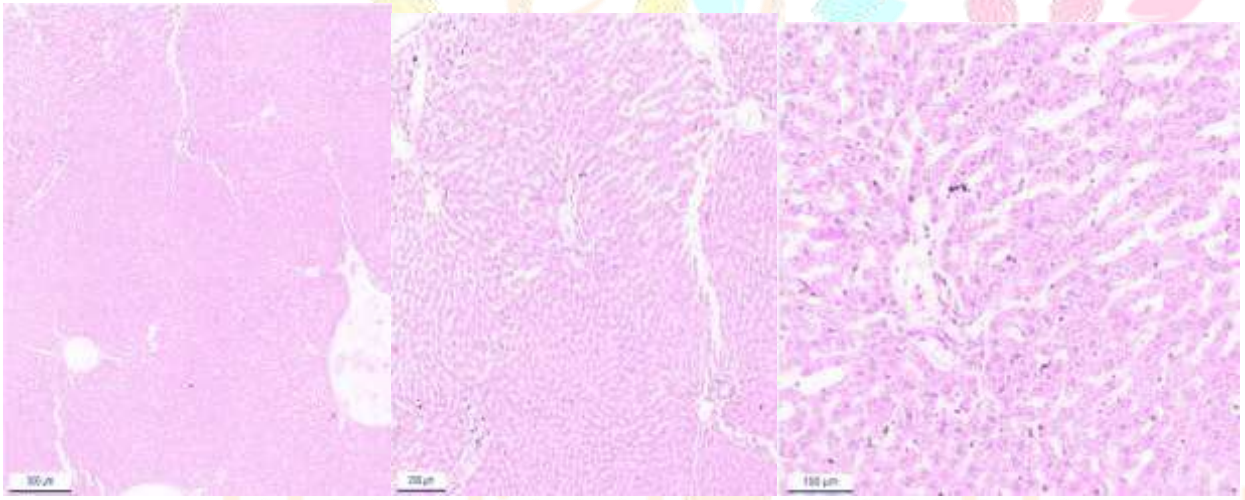
GROUP 3 – Chelidonium majus 200- Moderate (++) Degree of congestion , marked degree of bile pigment deposition .

Fig 7



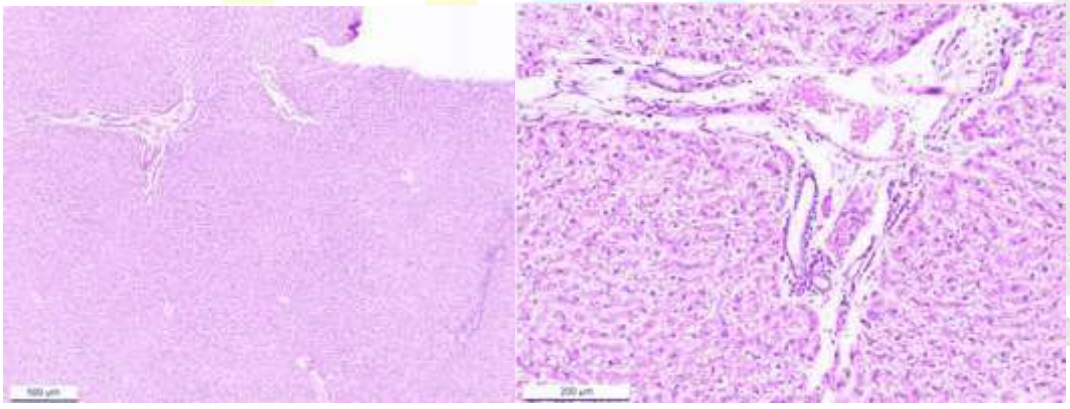
GROUP 4 – CROTALUS HORRIDUS 30 - No evidence of congestion , mild (+) degree of bile pigment deposition .

Fig 8



GROUP 5 – CROTALUS HORRIDUS 200- No evidence of congestion , minimal degree of bile pigment deposition .

Fig 9



GROUP 6 – PHENOBARBITAL - Multiple necrotic foci with mild peri – portal and peri – central fibrosis observed within the hepatic parenchyma .

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4. OBSERVATION AND RESULTS

The rats were divided into 4 groups and all were induced with hypothyroidism by using propylthiouracil. Then as per the group allotment, they were given the intervention medicines. When comparing the blood results of TSH, it is observed that the TSH values has been decreased after intervention in all the groups except the disease control group, which is the Group 1. In Group 4, there is mild reduction and in Group 2 and 3 there is significant reduction of TSH after medication. Hence, after intervention the TSH value in these hypothyroid induced rats were controlled with the help of our medications.

5. STATISTICAL ANALYSIS

DIRECT BILIRUBIN - The mean total bilirubin levels for each treatment group are displayed in this table. Significant liver damage was indicated by the highest mean bilirubin level (5.0600) in the disease control group (after phenylhydrazine induction). Every treatment group had significantly decreased bilirubin levels, but Chelidonium 30 (0.0633) and Crotalus Horridus 30 (0.0667) had the lowest values, indicating that these two were the most successful in reducing bilirubin. Phenobarbital, Crotalus 200, and Chelidonium 200, on the other hand, had somewhat higher mean values, suggesting less significant but still advantageous benefits. Therefore, the most successful therapies in this trial were Crotalus Horridus 30 and Chelidonium 30.

INDIRECT BILIRUBIN This data indicates that all treatments, both homeopathic and allopathic, significantly decreased the measured parameter (mean 5.5167), although phenylhydrazine induction significantly increased it. Stronger reactions were observed at greater potencies, with Phenobarbital having the highest mean effect (0.5233) across treatments, followed by Chelidonium 200 and Crotalus horridus 200. Crotalus 30 had no change and the least effect (0.0300). The findings indicate that phenobarbital and strong homeopathic remedies both successfully treated the created state, with consistent outcomes across repetitions.

TOTAL BILIRUBIN - Strong toxicity was shown by the high mean value (10.79) that phenylhydrazine induction produced. Phenobarbital (0.6467) had the strongest effect among the therapies, followed by Chelidonium 30 (0.5600) and Chelidonium 200 (0.4533). Crotalus 30 had the least amount of reaction (0.1200). The success of both homoeopathic and allopathic solutions was confirmed by the considerable reduction in toxicity observed in all treatments. Data dependability is supported by tight confidence intervals and consistent results, with the exception of Crotalus 200 and Chelidonium 30.

ANOVA - In every instance, the ANOVA summary spanning three datasets reveals a highly significant difference between the treatment groups. Compared to the "Within Groups" (0.004, 0.126, and 0.426), the "Between Groups" sum of squares (61.294, 69.750, and 268.391) is significantly larger, suggesting that treatment differences, not random error, are the main cause of the variation in outcomes. As demonstrated by the extraordinarily high F-values (34477.750, 1325.063, and 1510.882) and consistently.000 p-values (Sig.), the observed differences between group averages are statistically significant ($p < 0.001$). This confirms that the therapies, whether conventional or homeopathic, produced noticeable and quantifiable result.

6. DISCUSSION

In order to treat phenylhydrazine-induced hyperbilirubinemia, the current experimental study compared the effects of phenobarbital, a traditional allopathic hepatoprotective medication, with Chelidonium majus and Crotalus horridus. Phenylhydrazine effectively caused hyperbilirubinemia by significantly increasing total, direct, and indirect bilirubin levels, confirming its strong hemolytic and hepatotoxic effects through liver damage and red blood cell destruction. In one study, rats exposed to toxins showed reduced oxidative stress, tumors, and liver damage when treated to Chelidonium majus at 30C and 200C, with the latter showing somewhat better outcomes [10] . In a different study, According to Mitra et al. (1992), Chelidonium majus extract (125 mg/kg/day) stabilized membranes and controlled liver enzymes to prevent CCl_4-induced liver damage [9] .

The study was conducted at Daleview College of Pharmacy. animals that are housed in cages and fed regular meal pellets and drink. There are three rats in each of the six groups. A one-dram bottle with 0.5 ml pure water is used to take two pills of homeopathic medicine twice. Organon of Medicine aphorism 272 states that a similar globule broken with some milk sugar, dissolved in a big volume of water (§ 247), and well spun before each administration will provide a much more potent treatment for usage for several days. (A water dose of the drug was given.).

The study's blood parameters—direct, indirect, and total bilirubin—were assessed both before and after the medicine was administered. The findings indicated that whereas Chelidonium majus 30 was more significant for direct bilirubin, Crotalus horridus 30 was more significant for indirect and total bilirubin levels. "A properly selected homoeopathic remedy gently removes the disease without producing any new symptom," according to aphorism 157.

However, if the dosage is too high (or too weak) to generate a slight irritation, it is typical for a few hours.Aphorisms 161 and 247 state that aggravation does not happen when a correctly chosen homoeopathic medication is given in low potencies that are gradually increased and changed by bigger potencies [11,12]

7. CONCLUSION

The study's findings demonstrated that the homeopathic remedies Crotalus horridus 30C, 200C, and Chelidonium majus 30C, 200C, shown very good outcomes for treating hyperbilirubinemia brought on by phenylhydrazine. The treatment outcome was analyzed by comparing the levels of total bilirubin, direct bilirubin, and indirect bilirubin.

DIRECT BILIRUBIN – The group that received Chelidonium majus 30C showed the biggest decrease in direct bilirubin levels out of all the treatment groups. Chelidonium majus 30C may be slightly more effective in treating conjugated hyperbilirubinemia because of its propensity for hepatobiliary issues and its known liver-specific effects.

INDIRECT AND TOTAL BILIRUBIN – The best results were obtained with Crotalus horridus 30C. This points to a process that involves either stabilizing hepatocyte activity or improved hepatic clearance of bilirubin.

A statistical analysis was performed on the results. The results were significant, and the p-value was less than 0.01.

8. ETHICAL STATEMENT

This study was carried out in strict compliance with ethical guidelines and principles to safeguard the welfare and well-being of the animals involved. Ethical approval was obtained from the Dale View College of Pharmacy & Research Center under approval number **1118/PO/Re/S/07/CPCSEA**, following a comprehensive review and evaluation of the study methodology.

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