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Invivo Protective Effect of *Indigofera* on Hepatic Tissue

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ABSTRACT

Oxidative Stress is the primary cause of most of the diseases currently in the world. The human body is prone to many illnesses out of which oxidative stress plays a significant role in the causation of those. The Stress in the body releases free radicals and these free radicals react with the cell organelle and cause denaturation and break down. The generated free radicals should be fought to eliminate and prevent toxic effects. Many drugs are known to fight free radicals. Most of them had been scientifically evaluated and published for their *in-vitro* activity too. The investigations say that *in-vitro* antioxidant activity of the herbs does not similarly reflect *invivo*. There were differences in activity and significant deviations in the results that are shown in comparison to *invitro* and *invivo*. Thus, there is an urgent necessity to investigate the *invivo* antioxidant activity of herbs to make sure that the activity resembles and to find any variations when compared to *in-vitro* activity. This research protocol was framed out to screen for the *invivo* antioxidant activity of the ethanol extracts of the Indigofera *tinctora* leaves by estimating the serum parameters and correlating the results with the human body. Out of the values overall, the peroxidases were the ones that are significant participants in fighting the free radicals and in boosting the immune system. The ethanol extract successfully prevented the breakdown of the cellular structure of the liver cells, thereby protecting it and replenishing the enzyme levels by destroying the free radicals that are generated due to DMH.

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INTRODUCTION

Oxidative Stress is the primary cause of most of the diseases currently in the world. The human body is prone to many illnesses out of which oxidative Stress plays a significant role in the causation of those. The cell membranes are the most affected structures in the body due to oxidation and lipids, genetic material like DNA and RNA are other majorly affected organelles. The Stress in the body releases free radicals and these free radicals react with the cell organelle and cause denaturation and break down [1].

The generated free radicals should be fought to eliminate and prevent toxic effects. Many drugs are known to fight free radicals [2]. Most of them had been scientifically evaluated and published for their *in-vitro* activity too [3]. Out of those the synthetic drugs besides fighting the free radicals, they pose a severe threat to the body in view of their side effects and adverse effects. So, the alternatives for

these drugs were searched for, and herbs showed their potency and safety in this respect. Therefore, the antioxidant activity of herbs had to be investigated, and steps for proving the same are to be considered [4]. In this arena, many types of research had been performed, and the antioxidant activity of herbs had been determined and published.

The investigations say that *in-vitro* antioxidant activity of the herbs does not similarly reflect *invivo*. There were differences in activity and significant deviations in the results that are shown in comparison to *invitro* and *invivo* [5]. Thus, there is an urgent necessity to investigate the *invivo* antioxidant activity of herbs to make sure that the activity resembles and to find any variations when compared to *in-vitro* activity.

Indigofera tinctoria is a plant which is native of south Asia which contains abundant polyphenols and flavonoids [6]. The plant been tested for the antioxidant activity *in-vitro* and had proven to show a significant activity compared to the standard drug in the experiment. It was assayed using DPPH method *invitro* and showed 89% activity. There were investigations to estimate the phenol and flavonoid content of the plant leaves and were the ethanol extract was tested for antioxidant activity in nitric acid method too which showed significant results [7].

This research protocol was framed out to screen for the *invivo* antioxidant activity of the ethanol extracts of the *Indigofera tinctora* leaves by estimating the serum parameters and correlating the results with the human body [8].

METHODOLOGY

Extract Preparation

Leaves of plant *Indigofera tinctoria* were collected from a local garden in January and dried in an oven at 45^0c for 6hrs. After making sure the moisture is evaporated, the dried leaves were powdered using a rotary grinder, and the powder was sieved through mesh number 40 to achieve excellent and uniform powder [9]. This powder was used for extraction using Ethanol. 100gm of powder was taken and subjected to soxhlation until a clear liquid was run through the siphon. The extract was filtered, and the filtrate was evaporated to achieve a thick paste-like concentrated extract. It was stored and desiccated for future research [9].

Invivo Antioxidant activity on Liver

The *invivo* activity was performed on the albino rats that weigh between 0.15-0.20 kg were procured from an animal housekeeper in the city. The animals included both the sexes. They are maintained under the continuous circulation of conditioned air and was allowed to have free access for feed and water in the cage [10]. Coprophagy was prevented. Experiments on the animals were performed by using DMH method on the first day the 18animals were administered with DMH. After that, they were divided into three groups of 6animals in each group. A new group was created with 6animals and was not administered with DMG and was treated as a control group [11]. The first group of DMH groups were administered with normal saline, and the other two groups received the extracts at 250 and 500mg/kg body weight. The administration was continued for 30 days PO and DMH given to groups once every week in a month [12]. Body weights were noted before starting of the experiment and after ending of the 30 days of the tests. After the last day of the experiment, all the animals were left alone for 24 hrs, and they were sacrificed generously. Their livers were collected, and the antioxidant activity was determined by estimating the tissue parameters in the Liver [13].

Enzyme assay in the liver tissue

The extracted livers from the animals are appropriately marked and homogenized in a blender. The tissue homogenate was collected and was separated from the cellular material using a centrifuge. The liquid layer is collected and estimated for the below parameters. Catalases were estimated using the erythrocyte lysate assay method proposed by Sinha, 1972; Glutathione Peroxidase was assayed using the same method proposed by Rotruck et al., 1973, Glutathiones were estimated using Ellman method, Proteins and Superoxide dismutases were estimated using methods recommended by Lowry and Kakkar respectively.

RESULTS AND DISCUSSION

The ethanol extract of the plant *Indigofera tinctora* was investigated for its antioxidant activity in two doses 250 and 500mg/kg in Wistar albino Rats. The plant extract was proven for its hepatoprotective activity *invivo* in CCl_4 induced hepatotoxicity, and the research publications were made too. Based on this, the liver tissue was selected for the estimation of the antioxidant activity of the plant extract *invivo* by estimating the liver enzymes that are results of the free radicals in the cells.

Out of the parameters estimated in the tissue, Peroxidases are the major class of the enzymes which are responsible for fighting off the free radicals and were significantly lowered in the DMH treated groups with normal saline which was almost

Table 1: *In*- vivo antioxidant effect of Indigofera on hepatic tissue

Parameter	Normal control	Negative control	Extract 250mg/kg	Extract 500mg/kg
Catalase	40.41 ±0.62	27.89 ±0.93	23.34 ±0.85*	27.85 ±0.61
Superoxide Dismutase	4.892 ±0.738	1.724 ±0.063	2.556 ±0.071*	2.658 ±0.124*
Lipid Peroxidase	102.62 ±2.89	43.22 ±2.14	59.53 ±1.04*	60.31 ±1.19*
Protein (mg/g)	0.8431 ±0.0753	0.3462 ±0.0891	0.6714 ±0.0426*	0.7853 ±0.0872*
GSH	106.22 ±4.57	78.71 ±3.90	96.45 ±10.16*	105.22 ±12.04*
Glutathione Peroxidase	256.4 ±6.11	83.78 ±1.80	218.12 ±2.67*	242.93 ±13.68*

*P<0.0Dismutasesunits/min/mg protein; Catalases are expressed in moles of H2O2/min/mg protein; peroxidases are expressed in mg/g protein; GSH are expressed in mg/g of protein; Lipid peroxidases are expressed as MDA/mg of protein

reduced by 50% in the case of lipid peroxidases and 100% lowered in case of glutathione peroxidases. The enzyme levels were restabilized with extract-treated groups which are suggestive of the hepato-protective activity is due to the antioxidant activity of the extract.

Enzymes like catalases and dismutases were not revealed with the extract treatment. It was in contrast with the antioxidant activity of the extract *in vitro*. It showed better activity *in vitro*, but it was inefficient in successful replenishment of the catalases. It shows that there is a significant variation in the invitro and invivo activities.

The protein content in the normal saline-treated group was shallow, which is indicative of the fact that the free radicals generated by DMH denatured the cellular protein causing their break down. It resulted in int eh lowered value in the assay. Their level is replaced, and the proteins are prevented from breaking down by the treatment with the extracts. Extracts exhibited a dose based activity wherein 500mg/kg extract showed better activity in normalizing the enzyme levels in the tissue compared to its lower dose of 250 mg/kg.

The results were tabulated in Table 1, and it shows a significant difference in the *invitro* and *invivo* activities of the extract. Overall the mechanism of the hepatoprotective activity of the extracts was assumed due to the antioxidant activity of the extracts. This assumption was supported and claimed valid because the extracts normalized the antioxidant enzymes that are present in the liver cells, and they prevented the free radicals from causing Stress int eh hepatic cells.

Out of the values overall, the peroxidases were the ones that are significant participants in fighting the free radicals and in boosting the immune system. The ethanol extract successfully prevented the breakdown of the cellular structure of the liver cells, thereby protecting it and replenishing the enzyme levels by destroying the free radicals that are generated due to DMH.

CONCLUSION

Indigofera tinctoria was tested for the *invivo* antioxidant activity having an assumption that the *in-vitro* results have significant changes in the *invivo* environment due to many reasons. Also that the *invivo* experiments involve animals and the ethics committee limits the use of animals for experimental purposes. The facts that there is a change in the activity *in-vitro* and *in-vivo* there need to develop newer methods that simulate *invivo* animal models for experiments. So research needs to be conducted to establish models that duplicate and simulate animals models.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

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