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(RESEARCH ARTICLE)

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Screening of liver protective effect of herbal hepatoprotective product in ethanol induced hepatotoxicity in Wister rats

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Abstract

Background: liver is highly susceptible to the toxic and adverse effects of various metabolites and xenobiotics such as alcohol etc. Alcohol abuse leads to hepatotoxicity that is presented in the form of Alcoholic Liver Disease.

Aims and Objectives: The present study was conducted to evaluate hepatoprotective activity of polyherbal formulation. The test drug was studied in comparison with marketed formulations in ethanol induced hepatotoxicity in wistar albino rats.

Methods: Total 7 groups of animals were studied comparatively to evaluate efficacy of various formulations.

Results: It was found that all the formulations tested including all the dosages of Polyherbal Capsule significantly reduced levels of SGOT (Serum glutamic oxaloacetic transaminase), SGPT (Serum glutamate pyruvate transaminase), Alkaline phosphatase (ALP) and Total Bilirubin when compared to ethanol group. There was significant increase in total protein level in Ariliv Tablet, Silymarin and marketed formulation groups. Also, all the formulations tested effectively preserved the structural integrity of the hepatocellular membrane and liver cell architecture damaged by ethanol. When compared between formulation groups there was no statistical significant difference.

Conclusion: It can be concluded that Polyherbal Capsule possesses hepatoprotective activity in ethanol induced hepatotoxicity in rats. Polyherbal Capsule can be effectively used in hepatitis caused due to alcohol.

Keywords: Hepatoprotective; Ethanol; Polyherbal Capsule; SGOT; SGPT

1. Introduction

Liver is the largest organ in the human body. It performs more than 500 metabolic functions. Synthesis, storage, transformation and clearance of various chemical compounds and their metabolites are important metabolic functions performed by liver that make it highly susceptible to injury caused by any of them [1].

Alcohol liver disease is a highly pervasive ailment among human beings which environs a range of hepatic disorders starting from simple steatosis (fatty liver) to cirrhosis/liver failure [2]. Ethanol consumption enhances the ratio of NADH/NAD+ in hepatocytes which causes disruption of β-oxidation of fatty acids in mitochondria leading to steatosis. Alcohol also increases the lipid transport to the liver from the small intestine leading to enhanced mobilization of fatty acids from adipose tissue which is taken up by the liver [3]. This causes damage to cell membrane of hepatocytes leading to augmented levels of transaminases (Alanine aminotransferase (ALT) and AST (Aspartate aminotransferase)) in blood stream. Alkaline phosphatase (ALP) is also present in hepatocytes which come into the circulation indicating hepatic

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damage. Gamma-glutamyl transferase (GGT) has a key role in preserving intracellular homeostasis of oxidative stress which protects cells against oxidative damage. It is present in cell membrane and is set free in circulation when cell membrane is damaged. Glutathione (GSH) is a powerful antioxidant in our body which prevents damage by oxidative stress which is lowered by alcohol. Alcohol intake causes accumulation of oxidative stress markers (Glutathione reductase (GR) and malondialdehyde (MDA) which causes further damage to liver [4-5].

Chronic alcohol abuse can induce hepatotoxicity and jeopardize the nor- mal functioning of the liver. Ethanol-induced hepatotoxicity is mainly due to the toxic by-products of its metabolism, which in- duce oxidative stress [6]. According to World Health Organization's global status report on alcohol and health 2018, in India, 60% and 33.3% of death due to liver cirrhosis are because of alcohol consumption in males and females, respectively among 100,000 population above the age of 15 years [7-8]. This emphasizes the importance of in- vestigating potential hepatoprotective agents to prevent alcohol- induced hepatic damage.

Polyherbal Capsule used as a hepatoprotective agent for the management of Hepatitis and Alcoholic Liver disease. It contains Bhumyamalaki *(Phyllanthus niruri)* [9], Liquirice *(Glycyrrhiza glabra)*[10], Punarnava *(Boerhaavia diffusa)* [11], Bhringraj *(Eclipta alba)* [12], Tulsi *(Ocimum santum)* [13], Daruharidra *(Berberis aristata)* [14], Pippali *(Piper longum)* [15,16] extract. Most of these ingredients have demonstrated hepatoprotective activities in isolation.The present study was planned to evaluate hepatoprotective activity of Polyherbal Capsule in comparison with marketed Products in the ethanol induced hepatotoxicity in rats.

2. Material and methods

The study was conducted by Mprex healthcare Pune-411057. The material used for the study is given in table No.1. Institutional Animal Ethics Committee (IAEC) has approved the study in the meeting held on 30th Dec 2018.

2.1. Materials

Table 1 Materials used for the study

Drugs and Chemicals	Manufacture
Ethanol	Changshu Yangyuan Chemical, China
Silymarin	Silybon (Micro Labs LTD)
Marketed Formulation	Himalaya Herbals
Polyherbal Capsule (Low, Medium and High dosages)	Mprex healthcare, Pune

Table 2 Animals used for the study

Sr. No.	Name of animal	Weight range	Gender	Numbers to be used
1	Male Albino Wistar rats	200-250 g	Male	90

2.2. Methodology

Rats were divided into seven groups (n=7). Animals belonging to Group-I served as Normal control and received saline water daily for 14 days. Animals belonging to Group-II served as positive control and received only 50% Ethanol (2 ml) p.o. for 14 days. Animals belonging to Group-III served as Standard I and received Silymarin (25mg/kg body weight) + 50% Ethanol (2 ml) p.o. for 14 days. Animals belonging to Group-IV served as Standard II and received marketed formulation (54mg/kg body weight) + 50% Ethanol (2 ml) p.o. for 14 days. Animals belonging to Group-V served as Formulation low dose and received Formulation low dose (110 mg/kg body weight) + 50% Ethanol (2 ml) p.o. dose for 14 days. Animals belonging to Group-VI served as formulation medium dose and received formulation medium dose (220 mg/kg body weight) + 50% Ethanol (2 ml) p.o. dose for 14 days; Animals belonging to Group-VII served as formulation high and received formulation high dose (440 mg/kg body weight) + 50% Ethanol (2 ml) p.o. dose for 14 days . All the animals were weighed on Day 1, 7, 10 and 14. After the study period Animals were anaesthetized under light ether anesthesia. Blood was collected by retro orbital plexus puncture. Biochemical estimations of various

parameters like total proteins, total Bilirubin, SGOT, SGPT, and ALP were carried out. Animals were sacrificed and liver was removed, weighed and perfused in ice-cold saline solution for Histopathology studies [14-16].

3. Results

3.1. Effect of various Products on body weight in rats:

Table 3 Effect of various Products on body weight in rats

Groups	Body Weight (Mean ± SEM)			
	1st day	7th day	10th day	14th day
Normal	147±1.62	153±1.15	158±1.38	166±1.3
Ethanol	152±2.64	180±1.95##	212±0.98##	200±1.2##
Silymarin	149±2.64	155±1.05**	153±1.98**	166±0.9**
Marketed Product	150±2.65	156±1.55**	154±1.08**	157±1**
Product Low	155±1.64	155±0.85**	164±1.08**	165±1.7**
Product Medium	152±1.62	158±1.15**	161±0.88**	166±0.8**
Product High	153±1.62	163±0.85**	166±1.08**	169±0.7**

It is evident from the results of the study that the mean body weight of the rats in Ethanol group increased significantly over the period of 14 days when compared to normal group. No such significant weight gain was observed in any formulation group when compared with ethanol group. Even Between the formulation groups there was no significant difference observed (Figure 1).

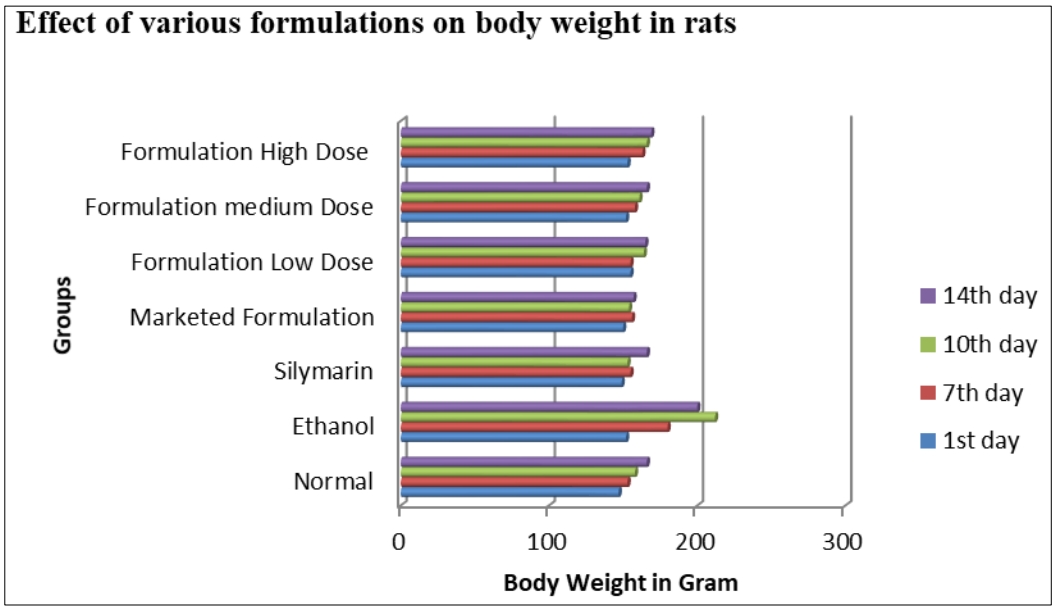

Figure 1 Effect of various Products on body weight in rats

3.2. Effect of various Products on Liver weight, SGPT, SGOT(U/L), ALP(U/L), Total Protein (g/dl), Total Bilirubin(g/dl)

Table 4 Effect of various Products on Liver weight, SGPT, SGOT, ALP, Total Protein, Total Bilirubin

Groups	Wt. of Liver in gm	SGPT(U/L)	SGOT(U/L)	ALP(U/L)	Total Bilirubin(g/dl)	Total Protein (g/dl)
Normal	2.894±0.83	51.05±0.4	22.1±0.152	71.73±0.31	1.552±0.196	4.55±0.074
Ethanol	5.344±0.99##	120.67±0.27##	91.9±0.322##	173.41±0.24#	5.852±0.18	2.00±0.086**
Silymarin	3.214±1.25**	55.57±0.25**	26.9±0.312**	73.72±0.24**	1.652±0.116**	4.5±0.073**
Marketed Product	3.264±0.87**	56.09±0.23**	29.45±0.582**	77.03±0.45**	1.992±0.14**	4.53±0.076**
Product (LD)	3.174±1.31**	58.09±0.22**	32.9±0.147**	75.65±0.23**	1.872±0.15**	4.37±0.111**
Product (MD)	3.074±1.2**	53.99±0.233**	27.4±0.142**	73.05±0.34**	1.682±0.14**	4.42±0.181**
Product (HD)	2.954±0.87**	53.29±0.24**	26±0.212**	69.89±0.27**	1.632±0.16**	4.51±0.091**

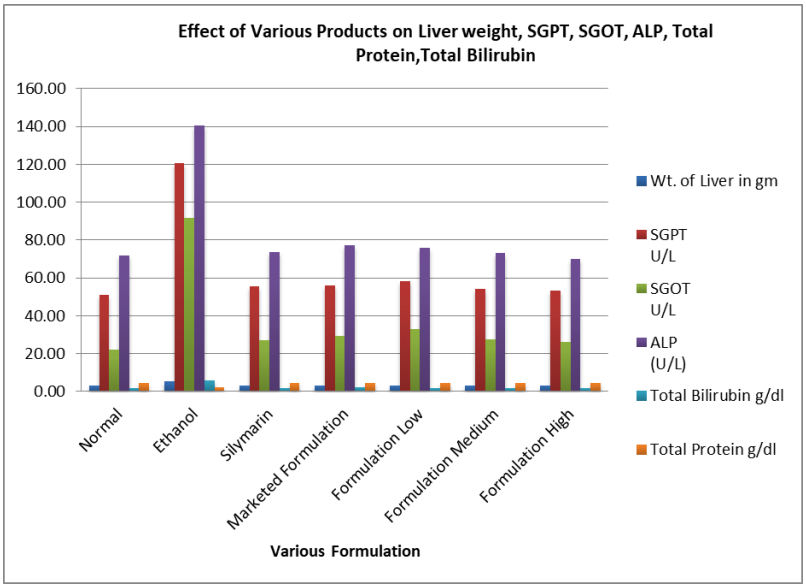

Figure 2 Effect of Various Products on Liver weight, SGPT, SGOT, ALP, Total Protein,Total Bilirubin

The results of the study indicate that liver weight in rats of ethanol group increased significantly when compared to normal group. This is in line with the fact that ethanol gives rise to fatty changes in the liver that leads to increase in the liver weight. No such significant increase in liver weight was observed in any formulation group when compared with ethanol group. When compared between the formulation groups there was no significant difference observed. SGPT level in ethanol group was increased to 120.67±0.27 which is highly significant when compared to normal group, indicating damage to the liver tissues. It is evident that all formulation groups successfully reduced the SGPT levels when compared to ethanol group. When compared between the formulation groups, no significant difference was visible. The above results show that, SGOT level in ethanol group is 91.9±0.32 which is remarkably greater than that of normal group (22.1±0.152). No formulation group shows such significant increase in SGOT level when compared to ethanol group. Between the formulation groups no significant difference was observed. Liver damage caused by ethanol is evident from the above results wherein ALP level is significantly increased in ethanol group (173.41±0.24) when

compared to normal group (71.73±0.31). All other formulation groups were successful to attain the normalcy in ALP level when compared to ethanol group. When compared between the formulation groups no significant difference was observed. Total bilirubin was increased to 5.852±0.18 in ethanol group. This five fold increase when compared to normal indicates significant liver damage due to ethanol. All other formulations significantly reduced total bilirubin level when compared with ethanol group. No significant difference was noted when compared between the formulation groups. It is evident from the study results that Ethanol markedly reduced total Protein levels (2.00±0.086) when compared to normal group (4.55±0.074). No formulation group reduced the Total Protein level when compared to normal group. When compared between the formulation groups no significant difference was observed [12-16].

3.3. Effect of various Products on Histopathological parameters in liver:

Table 5 Histopathological representation of liver in Ethanol induced liver injury in rat treated with Various Products (H&E stain, 40X)

Groups	Necrosis	Inflammation	Blood vessels	Hemorrhage	Fibrosis	Other
Normal control	Absent	Absent	Normal	Focal	+	--
CCl_4	+	++	Congested	+	Absent	--
Silymarin	Absent	Absent	Normal	Absent	Absent	Fatty change
Marketed Product	Absent	+	Dilated	Absent	Absent	--
Product Low	Absent	Absent	Normal	Absent	Absent	--
Product Medium	Absent	Absent	Dilated	Absent	Absent	--
Product High	Absent	Absent	Normal	Absent	Absent	--

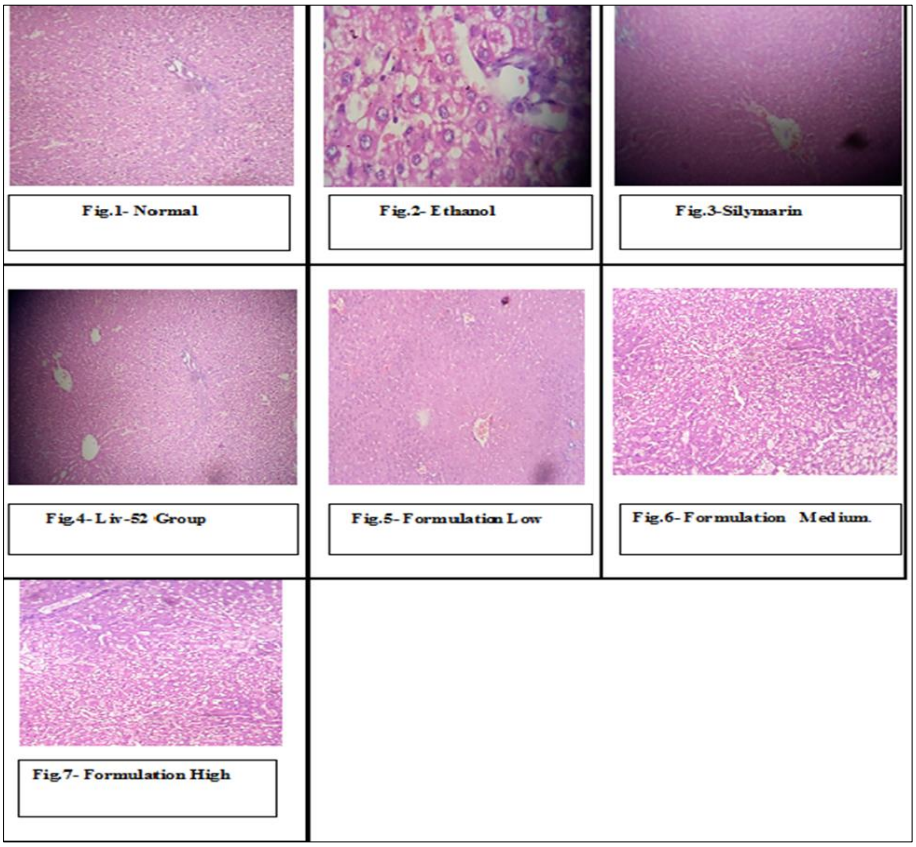

Figure 3 Histopathological representation of liver in Ethanol induced liver injury in rat treated with Various Product (H&Estain,40X)

On the Histopathological examination of liver tissues Normal control liver tissues showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. There were no signs of inflammation. Blood vessels were normal. Few signs of hemorrhage and mild fibrosis were also noticed that could be considered as an accidental finding. Whereas signs of toxicity such as necrotic cells, inflammation and hemorrhage were found in Ethanol induced liver tissue. It also showed congested blood vessels and the Fibrotic changes were absent. In a standard control group of Silymarin, no signs of toxicity were found. Blood vessels were normal, fibrosis was absent but fatty changes were evident. In the group of marketed formulation except mild inflammation and dilated blood vessels, no signs of toxicity were observed. Fatty changes were also absent. In all the groups of Polyherbal Capsule i.e. low, medium and high dose, no signs of toxicity were found. Fibrotic and fatty changes were also totally absent in all the three groups. Blood vessels in the medium dose group were dilated, which is also a finding with the marketed formulation group. But this cannot be taken as a very significant finding as there were no hemorrhage and fibrosis.

4. Discussion

In the modern era human beings are exposed to multiple chemicals or xenobiotics directly or indirectly that may cause injury to liver cells. Symptoms and clinically significant disease follows only when the damage leads to impairment of liver function. Jaundice or yellow discoloration of skin and mucus membranes, pruritus, severe abdominal pain, nausea, vomiting, continuous bleeding, skin rashes, generalized itching, weakness, severe fatigue, dark urine and light colored stool, varicose veins, weight loss or weight gain in short period, are some of the symptoms that usually appear after impairment of liver function. Hepatotoxicity may lead to various diseases like Hepatitis, Cirrhosis, hepatocellular carcinoma, Sclerosing cholangitis and Wilson's disease. Affected individual may land into acute or chronic liver failure due to hepatotoxicity and subsequent liver damage. Alcohol is one such xenobiotic responsible for liver damage that is consumed by millions of people daily. Alcoholic liver disease (ALD) develops in individuals consuming excessive alcohol, giving rise to various patterns such as hepatitis, fatty liver and cirrhosis. This is an extremely common disease with significant morbidity and mortality of hepatic origin, considered as one of the major issues in public health domain.

Lifestyle modification and nutritional support are the treatment strategies presently used in the management of ALD. There is no approved pharmacological therapy available for ALD. If patient lands into the liver failure, liver transplant is thought to be the only choice left.

UDCA is the only allopathic drug approved by US FDA for hepatoprotection or the treatment of primary biliary cirrhosis (PBC). Though UDCA is reported to have hepatoprotective activity, its use beyond PBC is unjustified because of observed findings like liver cell failure, mutagenic effects, hepatitis, ascites, immune suppression, increased association with hepatocellular carcinoma in PBC and even death. Moreover there is very narrow range between its therapeutic and toxic dose. Liver transplant indicated in End Stage Liver Disease due to hepatotoxicity also carries potential adverse outcomes. Graft rejection, vascular and biliary complications, sepsis, and short term survival rates are some of the confounding factors for acceptance of liver transplant. Besides its high cost and necessary ultra-modern nursing care makes it unsuitable option for general population. Therefore, there is immense need of safer and effective hepatoprotective agents that can prevent the damage, and improve the liver functions. In this context, a lot of attention has been focused on use of herbs that can provide hepatoprotective function with minimal side effects and better acceptability. Since thousands of years various herbs have been used in Traditional system of medicine like Ayurveda to treat jaundice and other liver disorders. Many of them have reported to have hepatoprotective activity and beneficial effects in liver disorders. Hence efforts are being taken to formulate drugs from these herbs to provide safe, cost effective, well tolerated and potential hepatprotective agent.

With this background, MPREX Healthcare has conceptualized and developed Polyherbal Capsule which is a polyherbal formulation of herbs with proven hepatoprotective activity. In the present study we evaluated the hepatoprotective activity of Polyherbal Capsule in comparison with Silymarin, a well-recognized hepatoprotective agent that is known to neutralize the toxic effects produced by CCl4, acetaminophen, ethanol and galactosamine induced hepatotoxicity models in rats. Polyherbal Capsule was also compared with one more polyherbal combination present in the market for the management of liver disorders. The activity of Polyherbal Capsule was studied in three doses- high, medium and low- in three different groups. All these formulations were compared with each other and with normal control and ethanol group. All the three groups of Test formulation significantly reduced the serum level of SGOT, SGPT, ALP and Total bilirubin. Total Protein levels were significantly improved in treatment groups. Mean body weight in rats did not increase significantly when compared to ethanol control group. In ethanol induced toxicity, resultant fatty changes in liver lead to increase in its weight. But in the groups treated with the formulations no significant increase in liver weight was observed.

On the Histopathological examination of liver tissues Normal control liver tissues showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. There were no signs of inflammation. Blood vessels were normal. Few signs of hemorrhage and mild fibrosis were also noticed that could be considered as an accidental finding. Whereas signs of toxicity such as necrotic cells, inflammation and hemorrhage were found in Ethanol induced liver tissue. It also showed congested blood vessels and the Fibrotic changes were absent. In a standard control group of Silymarin, no signs of toxicity were found. Blood vessels were normal, fibrosis was absent but fatty changes were evident. In the group of marketed formulation except mild inflammation and dilated blood vessels, no signs of toxicity were observed. Fatty changes were also absent.

In all the groups of Polyherbal Capsule i.e low, medium and high dose, no signs of toxicity were found. Fibrotic and fatty changes were also totally absent in all the three groups. Blood vessels in the medium dose group were dilated, which is also a finding with the marketed formulation group. But this cannot be taken as a very significant finding as hemorrhage and fibrosis were absent. Therefore, it can be stated that all the test formulation groups have been successful in regressing the pathological changes generated by ethanol. All the above findings suggest that high, medium and low dose formulation groups show significant hepatoprotective activity in terms of reduction in serum levels of liver enzymes such as SGOT,SGPT,ALP that are usually released in response to the damage to hepatic parenchyma. Lowering of Total bilirubin indicates improvement in Liver function. All these effects are highly significant when compared with Ethanol group, establishing heaptoprotective activity of all the formulations.

Though all the three groups of formulation exhibit significant hepatoprotective activity, we recommend medium dosage formulation for use in humans as it would be better tolerated and equally effective. All the ingredients of Polyherbal Capsule possess hepatoprotective activity individually, the hepatoprotective activity of the combination could be considered to be the synergistic effect of all the constituents [6-10].

5. Conclusion

It can be concluded from the results of the present study that Polyherbal Capsule possesses hepatoprotective activity for high, medium and low doses in Ethanol induced hepatotoxicity in rats. No signs and symptoms of toxicity in any group were observed. Though high dose and low dose formulations are effective as hepatoprotective agents, medium dose formulation of Polyherbal Capsule is recommended for potent hepatoprotective effect in humans.

Compliance with ethical standards

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Disclosure of conflict of interest

I declare no conflict of interest.

Statement of ethical approval

All the experiments were permitted and conducted as per the guidelines of Institutional Animal Ethical Committee (Approval no. CPCSEA/IAEC- 009/2022).

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