CHEMOPROTECTIVE EFFECT OF *BUCHANANIA LANZAN* SEED EXTRACT ON TOXICITY OF 5- FLUROURACIL INDUCED HEPATOTOXICITY IN RATS.

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Abstract*:* Objective-5-fluorouracil-induced (5-FU), an anticarcinogenic agent, is reported to have side-effects that include hepatotoxicity. The study objective was to investigate the protective effects of *Buchanania lanzan* on 5-FU-induced hepatotoxicity. Material and Methods- Male Wistar rats were assigned to seven groups. Weighing 220–250 g, were included in the study. The animals were housed under adequate moisture and light conditions, at a suitable room temperature, and were provided with sufficient water and food until the day of the experiment. The control group received distilled water for 21 days. The second group of animals injected only with 5-FU (20 mg/kg i.p) on day 17 to 21. Third, fourth group of animals administered with extract of *Buchanania lanzan* (200 and 400 mg/kg p.o.) and injected with 5-FU on day 17 to 21. Fifth and Sixth group of animals only injected with extract of *Buchanania lanzan* (200 and 400mg/kg p.o.) and seventh group of animals injected with standard Silymarin for 21 days respectively and injected with 5-FU (20 mg/kg i.p) on day 17 to 21. On day 22, the rats were decapitated, and blood and hepatic tissues were taken. *Buchanania lanzan* was observed to have a protective effect on 5-FU induced Liver toxicity. Results of histopathological examination also revealed multifocal moderate hepatocellular vacuolation (macro vesicular) in 5-FU treated rats while prior treatment with Buchnania lanzan for 21 days showed focal mild hepatocellular vacuolation in liver. Conclusion-In this study it was determined that the *Buchaniana lanzan* have protective effects on 5-FU-induced liver toxicity.

Index Terms – Buchanania lanzan **spreng seed, Aqueous ethanolic extract, 5-Flurouracil, Silymarin, Hepatoprotective, Histopathology.**

1.0 INTRODUCTION

5-fluorouracil (5-FU), a fluorinated pyrimidine, is classified as an antimetabolic agent and influences the synthesis of DNA and RNA in normal and tumor cells. The majority of 5-FU is abolished through liver metabolism and only a small portion is removed from the body via kidney excretion. 5-FU is widely used in chemotherapy for various cancers (Longley *et al*, 2003). As a fluoropyrimidine antimetabolite agent, it plays an important role in the treatment of colon, breast, gastrointestinal, head, neck, and pancreatic cancer. In addition, it has hepatotoxic effects, with increased aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activity in tissue (Volkan, 2017; and Ray, et al, 2007).

Liver is one of the largest organs in the human body and chief site for intense metabolism and excretion. Liver diseases are one of the major health problems in the world. These are caused by toxic chemicals, autoimmune disorders, infections and excess consumption of alcohol. The hepatotoxic chemicals can induce lipid peroxidation and oxidative damages. It is involved in almost all the biochemical pathways to growth, fight against the disease, nutrient supply, energy provision and reproduction. (DeLeve et al, 1995 and Farrel, 1998).

Buchanania lanzan spreng (locally called as Chironji), a member of family Anacardiaceae is a commercially useful tree species found in several areas of India. The plant has well-known traditional uses and its seeds are used as expectorant and tonic. The oil extracted from kernels is applied on skin diseases and also to remove spots and blemishes from the face. The root is used as expectorant, in biliousness and also for curing blood diseases. The juice of the leaves is digestive, expectorant, aphrodisiac and purgative. The rhizome of *B. lanzan* finds an important place in indigenous medicine as an expectorant, diuretic and carminatives. It is also found to have anticancer, antihypertensive, larvicidal and anti-diabetic activities. (Achuthan et al, 1997; and Choochote et al 1999). It is a commercially useful tropical plant. Chironji tree is a medium evergreen deciduous tree, growing 50 ft. tall. It bears fruits each containing a single seed, which is a popular edible nut, known as chironji. It is common in India mostly in eroded lands. It has tickly leathery leaves which are broadly oblong, with blunt tip and rounded base (Dai et al 2002; and Kumar et al, 2007). Silymarin was used as reference standard.

1.0 MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material:

Buchanania lanzan seeds were collected from the surrounding area of rural Pune during September 2018. The plant was identified and authenticated by M/s. Shamantak Enterprises, Dr. Gautam, Botanist, Pune, India.

2.2 Preparation of Plant Extract:

A weighed quantity (50g) of the air-dried powdered seeds Buchanania lanzan was drawn and then it was extracted with 90% ethanol in a Soxhlet extractor. The hydroalcoholic extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50˚ C to get a solid residue. Different concentration (200mg/kg, 400mg/kg p.o.) of hydroalcoholic extract of seeds *of Buchanania lanzan* was given according to body weight of animals.

2.3 Animals:

The study was undertaken at the AISSMS College of Pharmacy, Pune. The Institutional Animal Ethical Committee approved the protocol (CPCSEA/IAEC/PC-07/01-2K18) for the study. Wistar rats of both sexes (200-250g) were used. They were maintained at 25±2º C and relative humidity of 45 to 55% and under reversed light dark cycle (12 h light: 12 h dark cycle) (Johansen *et al*, 2008). The animals had free access to food and water ad libitum throughout study. All experiments were carried out between 9:00 – 16:00 hours.

2.4 Phytochemical Analysis:

The extract was subjected to preliminary phytochemical screening for phytochemical constituents such as Flavonoids, Tannins, Alkaloids, Glycosides, Triterpenoid, Saponins, Sterols, Carbohydrates,Phenolic compounds, Gum and Mucilag (Agrawal et al, 2007)

2.5 Experimental Design:

Forty-two male Wistar rats were divided into seven groups, six in each. Group 1 was served as normal control which was given with distilled water only. Group 2 treated with an in dose of 5-FU on 17-21 day of experiment. Group 3 treated with an oral dose of 200mg of extract +20mg/kg of 5-FU on 17-21 days. Group 4 treated with an oral dose of 400mg of extract +20mg/kg of 5-FU on 17-21 days. Group 5 treated with an oral dose of 200mg of extract for 21 days. Group 6 treated with an oral dose of 400mg of extract for 21 days. Group 7 treated with an oral dose of standard drug 140mg/kg of Silymarin + 20mg/kg of 5-FU on 17-21 days.

At the end of study, on the day 22, the rats were anesthetized, blood sample were taken by retro orbital method and the animals were sacrificed. Blood samples were centrifuge at 1500rpm (Micro centrifuge) for 15 minutes within 1 hr. of collection to obtain serum samples, which were immediately analyzed for estimation of Serum alanine aminotransferase (ALT), Serum aspartate aminotransferase (AST), Serum alkaline phosphatase (ALP), Serum total bilirubin (BILT), Serum albumin (ALB) and Serum total proteins (TP) by using standard kit (Biolab, Diagnostics, Mumbai). A histopathological examination of liver was performed to analyzed morphological analysis.

2.9 Histopathology of Liver:

Formalin fixed Liver tissues were trimmed and processed. Tissue processing was carried out to dehydrate in ascending grades of alcohol, clearing in xylene and fixed in paraffin wax. Paraffin wax embedded tissue blocks were sectioned at 3-5 µm thickness with the Rotary Microtome. Slides were stained with Hematoxylin & Eosin (H & E) stain. The prepared slides were examined under microscope to note histopathological lesions, if any. Distribution of the lesions was recorded as focal, multifocal and diffuse.

2.10 Statistical Analysis:

Statistical analysis was carried out using GraphPad Instat 3. All of the data is shown as the mean ± standard error of the mean (S.E.M) and were analyzed using one-way analysis of variance (ANOVA). Significant differences between the estrous control and experimental groups were determined using Tukey-Kramer test all comparison test, $P<0.001$ was considered significant.

3.0 RESULTS

3.1 Biochemical Parameters

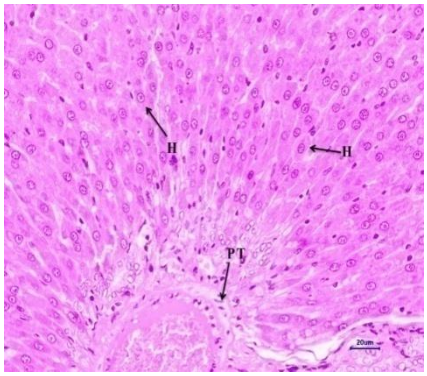
The Buchanania lanzan extract was effective in protecting liver against the injury induced by 5-FU in rats. Biochemical parameters like ALT, AST, ALP levels in serum and Total Bilirubin are significantly increased whereas Albumin and Total protein are significantly decreased compared with Control group. Treatment with 200mg/kg *Buchanania lanzan* daily for 21 days had no significant difference compared with Control group. Treatment with 400mg/kg Buchanania lanzan daily for 21 days had significantly increased like ALT, AST, ALT and Total Bilirubin and Albumin and Total protein significantly decreased as compared to Control group. Treatment with Standard group daily for 21 days had significantly increased compared with Control group **(Table 1).**

PARAMETERS	CONTROL	5FU	200mg+5FU	400mg+5FU	200mg	400mg	STD+5FU
ALT(SGPT) U/L	0.060±0.0013	0.095±0.0008	0.061±0.0017**	0.096±0.0016***	0.066±0.0011ns	0.087±0.0010***	0.081±0.002***
AST(SGOT) U/L	0.076±0.002	0.098±0.002	0.077±0.0014***	0.099±0.0016	0.077±0.0016***	0.079±0.0011***	0.095±0.0008***
ALBUMIN g/dL	1.814±0.019	1.881±0.1253	1.811±0.121	1.785±0.069 ns	1.813±0.067*	1.818±0.082	1.821±0.1507
TOT. PROTEIN g/dL	17.25±0.166	17.51±0.245	17.28±0.143**	12.49±0.166***	16.38±0.284***	17.43±0.258***	17.28±0.179
TOT. BILI mg/dL	0.621±0.023	0.6541±0.011	0.620±0.011***	0.666±0.011***	0.623±0.012***	0.646±0.011***	0.646±0.012***
ALP U/L	282.4±6.375	1215.1±48.51	284.1±0.298***	1228.8±13.70***	710.56±5.119***	811.9±4.750***	295.5±6.710***

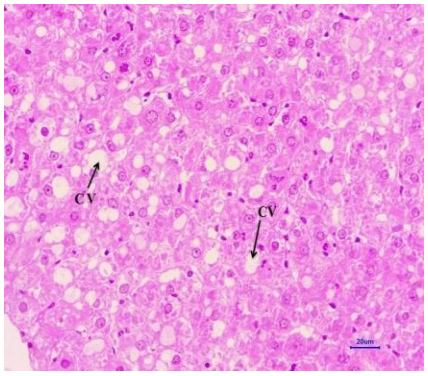
Results are expressed as mean ± SEM (n=6) Data was analyzes by one- way analysis of variance (ANOVA) followed by Tukey- Kramer's test. *p<0.05, **p<0.001, ***p<0.001.

3.2 Histopathological Study:

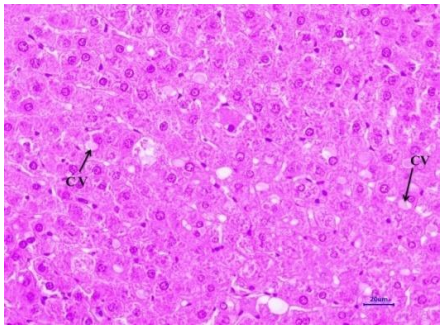
Liver of rats of control group did not reveal any lesion of pathological significance. Rats treated with 5-flurouracil showed multifocal moderate hepatocellular vacuolation (macro vesicular) in liver. Rats treated with 5-flurouracil with standard drug showed focal mild hepatocellular vacuolation (macro vesicular) in liver. Rats treated with 5-flurouracil with test drug at 200mg/kg and 400 mg/kg showed focal mild hepatocellular vacuolation (macro vesicular). Rats treated with test drug at 200 mg/kg and 400 mg/kg did not show any lesion of pathological significance in liver. Treatment of standard and test drug at 400 mg/kg reduced the adverse effect incurred due to treatment of 5- fluorouracil. All the Histopathology study images are mentioned **Figure 1.**



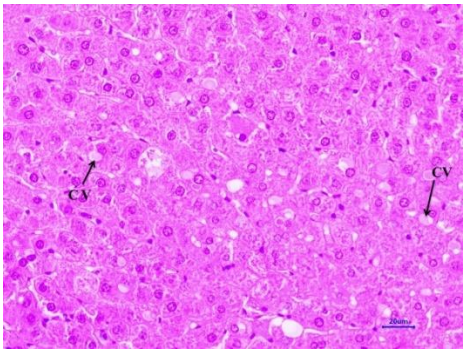
1. CONTROL



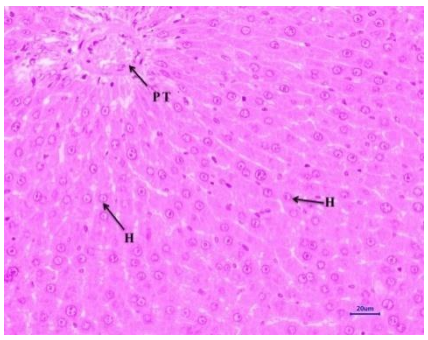
2. 5-FU



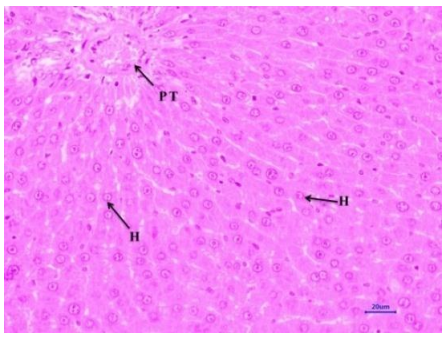
3. 200mg/kg *B. lanzan* + 5-FU



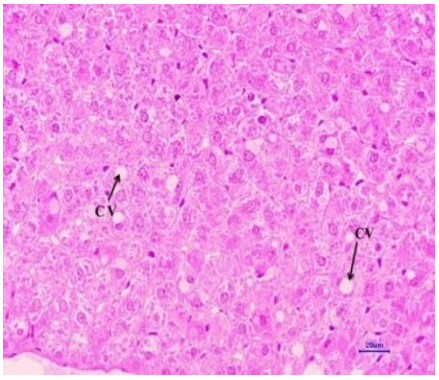
4. 400mg/kg *B. lanzan* + 5-FU



5. 200mg/kg *B. lanzan*



6. 400mg/kg *B. lanzan*



7. 5-FU + STD

Fig. 1 Histopathological examination of Liver of rats in Hepatotoxicity study.

4.0 DISCUSSION

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges, like xenobiotics, drugs, viral infections and chronic alcoholism. If during all such exposures to the above-mentioned challenges the natural protective mechanisms of the liver are overpowered, the results is hepatic injury. Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition, serum level of many biochemical markers like AST, ALT, Bilirubin, alkaline phosphatase is elevated (Setty et al, 2007).

Aspartate and alanine aminotransferases are normally localized within the cells of the liver, heart, kidney, muscles and other organs. The enzymes are of major importance in assessing and monitoring liver cytolysis. Their presence in the serum may give information on organ dysfunction (Yakubu et al, 2005).

ALT is more specific cytosolic enzyme for liver, whereas AST is localized in cytosol and mitochondria that are released into circulation in the early phase of injury. Prolonged destruction in hepatic cells results in more hepatic releases to exacerbate hepatic dysfunction and causes an elevation of ALP, Bilirubin in serum (Bhadauria et al, 2010).

A widely used chemotherapeutic agent, 5-FU, has proven efficacy in human malignancy. However, its clinical utility is inhibited by hepatotoxic side effect (Skretkowicz et al, 1996; El-sayyad et al, 2009). Chemotherapy is the main treatment option for cancer patients, but its therapeutic use is limited due to severe clinical side effects. (Ramadori et al, 2010; and Naidu et al, 2009).

Enzyme level such as ALT, AST are often used to assess hepatic damage. Liver injury causes membrane damage or necrosis, which allows intracellular enzymes to circulate and be detected in serum. Total protein, Total bilirubin, ALP level is also associated with liver cell function (Shehab et al, 2015; and Bagban et al, 2012).

In conclusion, *Buchanania lanzan* treatment can mitigate live damage after 5-FU- induced liver toxicity in rats. The protective roles of *Buchanania lanzan* could improve restoration of biochemical oxidative enzymes and antiapoptotic and liver cells. This may occur due to the antioxidant effects of *Buchanania lanzan*. Therefore, our experimental results suggest that *Buchanania lanzan* might potentially be protective agents for 5-FU-induced liver toxicity.

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