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HEPATOPROTECTIVE ROLE OF ALOE VERA JUICE ON ANTITUBERCULOSIS DRUGS ETHIONAMIDE AND PARA AMINO SALICYLIC ACID INDUCED HEPATOTOXICITY IN SPRAGUE-DAWLEY RATS

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ABSTRACT Fresh *Aloe vera* plant leaves were brought from botanical garden and sample was indentified and brought to the laboratory in the Department of Zoology, Patkar-Varde College, Goregaon (W), Mumbai. 50 grams of leaves were then grounded with 50ml of distilled water in sterilized pestle and mortar. The yield will be calculated based on weight of the extract compared to the weight of the pulp of the leaves. Forty eight (48) *Sprague- dawley* rats (average weight 150 - 250 g) of either sex were used for the experiment. The drugs ETH and PAS drug and *Aloe vera* juice were given to respective groups daily for 28 days. At the end of study various biochemical parameters were analyzed from serum such as, Bilirubin, ACP, ALP, SGOT (AST), SGPT (ALT), HDL Cholesterol, Total Cholesterol, and TGL. Liver tissues were analyzed for Histopathology. The data was statistically analyzed by one way analysis of variance (ANOVA). The value $p< 0.05$ considered as significant. Administration ETH and PAS in *Sprague-dawley* rats showed hepatotoxicity in test groups which was confirmed by biochemical parameters and histoarchitecture examination of liver. The rats orally administered of *Aloe vera* juice in combination with the ETH and PAS or independently found to be effective in lowering the elevated levels of bilirubin, ACP, ALP, ALT, AST, HDL to approximate normal level in ETH and PAS treated rats and by elevating the level of TGL. From the histopathalogical study it was also found that ETH and PAS could induce hepatitis through increases in oxidative stress, liver injury, and liver histopathology. *Aloe vera* juice in acts as hepato-protective agent and recovered the liver to its normal architecture. Thus current research implies the functional potential of *Aloe vera* juice in hepatotoxicity induced rats.

KEYWORDS : Aloe vera, ETH, PAS, Histoarchitecture, Liver. Rats.

INTRODUCTION

The liver is an important vital organ of the body to maintain metabolic functions and detoxification and elimination of xenobiotics, drugs and chronic alcoholism [1]. The liver plays an astonishing role in the maintenance, performance and regulation of the homeostasis of the body. It is involved in almost all biochemical pathways like carbohydrates, proteins, fat metabolism, secretion of bile and storage of vitamins for growth, nutrient supply providing energy to the cell and fighting against bacterial and viral diseases [2]. Thus, the liver plays a crucial role in the maintenance of a healthy liver and well- being [3].

Hepatotoxicity refers to harm to the liver by exposure to chemicals, xenobiotics, drugs, chlorinated solvents, food additives, fungal toxins, environmental toxicants and radioactive isotopes [4]. The chemicals that are used in chemical industries and laboratories and natural products used in herbal remedies can also cause hepatotoxicity. Those substances which are responsible for the cause of liver injury are called hepatotoxins. The liver is an important organ for metabolism and drug elimination [5]. Drug -induced liver disease is still a worldwide concern which limits the therapy and drug use. Nearly 2 % of the cases of jaundice in hospitalized patints are drug induced. Around quarter of cases with great severity of hepatic damage are of drug related. Now a day more than 900 drugs are present in the market have been implicated in causing liver injury and it is most common reason for a drug to be withdrawn from the market [6]. Drug-induced liver injury is responsible to cause 5% of all hospital admissions and 50% of all acute liver failures. More than 75 percent of cases of idiosyncratic drug reactions result in liver transplantation or death [7].

Mycobacterium tuberculosis is the infectious agent that causes tuberculosis (TB). Despite medical advances, tuberculosis remains fatal and is the leading cause of human death in many countries. Every second person in the world is infected with tuberculosis. The estimated number of new cases of tuberculosis every year around the world is around 9.6 million. Approximately one -third of the world's population is currently infected with tuberculosis and up to 10% of these will develop active TB, causing 1.6 million deaths per year [8]. It has been studied that the development of MDR-TB is due to misuse of proper antibiotic treatment by patients and lack of attention focused on these patients. The very high incidence of MDR-TB has led to the use of second-line tuberculosis drugs.

Ethionamide (eth eye on a mid) is the most commonly used drug which shares similarities with Isoniazid in terms of structure and anti-mycobacterial function. The daily oral dose of Ethionamide is 250 mg/kg and can be increased to 1 gram if well tolerated by the patient. Some cases of ethionamide-induced hepatotoxicity have been severe and harmful cases have also been reported [9]. Para-aminosalicylic acid (PAS) was the first antibiotic found to be effective in the treatment of tuberculosis in the 1940s[10]. PAS treatment is uncommon and highly drug-resistant strains have limited resistance to this drug. Thus, PAS became the principal second-line agent for the treatment of MDR-TB [11]. Hepatotoxicity is one of the most frequent and serious adverse effects of anti-TB drugs and can reduce treatment effectiveness by compromising treatment regimens[12,13].

Hepatoprotective activity refers to protection of the liver from hepatotoxins or counteracting changes in antiradical defense mechanisms [14]. Plant extracts may be the best source of such antioxidants and may mediate hepatoprotective activity. Many liver-protective chemical constituents such as coumarins, phenols, monoterpenes, alkaloid glycosides and xanthines are found in plants [15].

Several medicinal plants used traditionally for thousands of years are present in the herbal preparations of the Indian traditional health care system. Today, about 80% of the world population depends on botanical agents as medicines to meet their health issues [16]. In developing countries, traditional plant remedies are widely used to treat various diseases. Many varieties of plants have been used to treat a variety of diseases including hepatoprotective potential [17]. Nowadays, dietary supplements and herbal remedies have increased the interest of researchers in treating a variety of diseases. In India, more than 40 polyherbal commercial formulations reputed to have hepatoprotective action are being used[18,19].

More than 500 species of aloe are known, but Aloe vera is recognized as the "true aloe vera" for its widespread use and purported healing powers [20]. *Aloe vera* has been used for many centuries for its medicinal and therapeutic properties. Aloe juice has been used for centuries as a laxative and medicinal cleanser [21]. Many of the health benefits associated with *Aloe vera* are attributed to promoting wound healing, antifungal activity, hypoglycemic or antidiabetic effects, and anti-inflammatory, anticarcinogenic, immunomodulatory, and gastroprotective properties[22].

MATERIALS AND METHODS

a) Collection and Identification:
Fresh Aloe vera plant leaves were brought from botanical garden and sample was indentified and brought to the laboratory in the Department of Zoology, Patkar-Varde College, Goregaon (W), Mumbai. *Aloe vera* plant identified by reviewing the literature and the final identification and authentication was done at Department of Botany, St Xavier,,s College (autonomous) Mumbai, India.

b) Preparation of Crude Extract:
Fresh *Aloe vera* leaves were rinsed 2-3 times in the tap-water. 50 grams of leaves were then grounded with 50ml of distilled water in sterilized pestle and mortar. The yield will be calculated based on weight of the extract compared to the weight of the pulp of the leaves in a sterile container and keep at-20 ^{0}C till further use.

c) Purchas of drugs
The drug ETH (Ethionamide) (Macleods Pharmaceuticals Ltd) and PAS (Para-aminosalicylic acid) (Lupin Ltd) were purchased following the Prescription of Physician by the medical practitioner, from New Krishna Medicos, Shop No. 3, Salim Estate Near Times Square, opposite Kanakia Seven, Marol, Andheri,(E), Mumbai, India.

d) Experimental Design
Forty eight (48) *Sprague- dawley* rats (average weight 150 - 250 g) of either sex were used for the experiment. They were purchased and procured from the National Toxicological Centre, APT Testing & Research Pvt. Ltd. (ATR) Pune. The experimental study was approved by Ethical committee at APT Research Foundation, Pune prior to the experimentation (CPCSEA NO. RP 01/2223 dated 11/June/2022).The animals were acclimatized, maintained and housed in APT laboratory for a week.

The controlled humidity and temperature at 22+3^{0}C, humidity 50-60 %, and illumination cycle set to 12-hlight/12 hrs dark cycle was also maintained. Six rats per cage were housed in polypropylene cages with stainless steel grill top, facilities for commercial Pallet food and water bottle with ad-libitum, and bedding of clean paddy husk.

Table 1. Showing dose level of Aloe vera, ETH and PAS in different groups of Sprague- dawley rats

Groups (n=6)	Treatment
Group-1	Animals fed with rat pellets and ordinary water
Group -2	ETH(132 mg/ 70kg, p.o) for 28 days
Group -3	PAS(400 mg/70kg, p.o) for 28 days
Group -4	ETH (132 mg/kg, p.o) + PAS(400 mg/ 70kg, p.o) for 28 days
Group -5	ETH (132 mg/kg, p.o) + Aloe vera juice (50 ml/70kg, p.o) for 28 days
Group -6	PAS(400 mg/kg, p.o) + Aloe vera juice (50 ml/70kg, p.o) for 28 days
Group -7	ETH (132 mg/70kg, p.o)+ PAS(400 mg/70kg, p.o)+ Aloe vera juice (50 ml/70kg, p.o) for 28 days
Group -8	Only Aloe vera juice (50 ml/70kg, p.o) for 28 days

e) Administration of Test Article
The test article at the above concentration was administered to each rat by a single oral gavage. The animals were dosed using a stainless steel intubation needle fitted onto a suitably graduated syringe. The dosage volume administered to individual rat was adjusted according to its most recently recorded body weight. Animal weights were determined weekly along with food consumption. Animals were randomly divided into following groups containing 6 animals (3 males and 3 females) in each group. Test drug and inducers were given to respective groups as indicated in the table daily for 28 days. At the end of study various biochemical parameters were analyzed from serum such as, Bilirubin, ACP, ALP, SGOT (AST), SGPT (ALT), HDL Cholesterol, Total Cholesterol, and TGL.

f) Statistical analysis:
The data was statistically analyzed by one way analysis of variance (ANOVA). The value ($p< 0.05$) considered as significant. Statistical analysis: ANOVA followed. The p-value is ($P < .00001$) the result is no significant.

g) Biochemical assay
Blood samples of the above groups were taken after 28th day by heart puncture for estimation of liver functional test. Assessment of liver damage were done by biochemical investigations of Serum glutamic-pyruvic transaminase (SGPT) and Serum glutamic–oxaloacetic transaminase (SGOT) by [23], Serum bilirubin (Bil) by [24], Serum alkaline phosphatase (ALP) by [25], Cholesterol and HDL High density lipid by [26], Triglysceride by [27] and Acid Phosphatase by [28].

h) Histopathological analysis
The liver tissue was dissected out and fixed in 10% formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin. Five micron thick sections were prepared and then stained with hematoxylin and eosin (H–E) dye for photomicroscopic observation, including cell necrosis, fatty degenerative changes, hyaline regeneration, ballooning degeneration as proposed by [29] and histological structure of liver tissue were examined under the Biological digital microscope Motic B1 Series.

RESULTS AND DISCUSSIONS:

Table-2: Showing the mean concentration of Liver Serum Biochemistry of Effect of Aloe vera joice and drugs Ethionamide and Para amino salicylic acid, on Sprague- dawley rats

S. No.	Group		T. Bili	ACP	ALP	SGPT	SGOT	HDL.C	TGL	T.Chol
			mg/dL	IU/L	IU/L	IU/L	IU/L	mg/dL	mg/dL	mg/dL
1	A- NC	Mean	0.42	0.06	362.40	67.89	188.26	10.63	99.33	72.19
		SD	0.13	0.007	107.74	23.48	64.61	8.62	16.70	10.36
2	B- ETH 132 mg/kg	Mean	0.53	0.06	378.54	95.16	212.64	13.45	107.56	69.45
		SD	0.06	0.007	124.47	8.73	64.52	5.68	41.69	18.79
3	C- PAS 400mg/kg	Mean	0.53	0.06	383.37	90.29	217.98	8.66	119.50	74.36
		SD	0.12	0.007	84.56	12.66	26.37	2.52	27.28	10.64
4	D- ETH+PAS 90 mg/kg	Mean	0.53	0.07	410.95	97.24	239.73	12.60	138.06	61.59
		SD	0.09	0.007	185.84	13.32	77.41	8.70	19.71	13.15
5	E- ETH+ Aloe vera Juice 90mg/kg	Mean	0.52	0.05	307.52	89.26	194.40	13.03	136.00	75.93
		SD	0.07	0.007	109.42	12.20	66.30	6.13	25.50	23.34
6	F- PAS+ Aloe vera Juice 90 mg/kg	Mean	0.46	0.05	398.58	63.54	180.68	36.37	127.59	87.43
		SD	0.11	0.007	93.15	11.96	66.37	5.68	18.94	16.37
7	G- ETH+PAS+ Aloe vera Juice 90mg/kg	Mean	0.53	0.05	346.89	67.39	185.89	22.54	109.20	76.88
		SD	0.08	0.007	127.47	14.25	40.51	8.86	22.51	13.46
8	H- Only Aloe vera Juice 90mg/kg	Mean	0.51	0.04	354.28	68.09	179.54	23.75	115.27	70.09
		SD	0.10	0.007	122.57	7.83	7.38	8.88	18.86	7.75

*Each value is the mean of 8 determinations.
Bilirubin : Serum Bilirubin mg / dl
ACP: Serum Acid Phosphatase IU / L
ALP : Serum Alkaline Phosphatase IU / L
SGPT : Serum Glutamic Pyruvic Transaminase IU / L
SGOT : Serum Glutamic Oxaloacetic Transaminase IU / L

HDL Cholesterol TC: Total Cholesterol TGL: Triglycerid

The experiment was conducted up to 28 days. There was no mortality was noted in control and experimental groups. After 28 days the animals were sacrifices and blood, is withdrawn by heart puncture for estimation of liver functional test, as per the guidelines. The body weights and relative liver weights were estimated by dissecting the liver to calculate the difference in weights of liver in control and

experimental groups. The rate of food consumption was also calculated in the interval of every 7 days up to the end of the study.

The mean rate of food consumption (R=Remained, C=Consumed, C/A= Consumed / Animal Quantity of Food Given: 100) was calculated in every week in control and experimental group. In normal control group the rate of food consumption was fond to be (R= 38.50 gm ± 4.95; C=61.50 gm ± 4.95 and C/A = 20.50 gm ± 1.65). In experimental groups, the minimum food consumption was noted in group treated with PAS+ *Aloe vera* juice (R=60.25gm ± 6.72; C= 39.75 gm ± 6.72 and C/A = 13.25gm ± 2.24), whereas the maximum food consumption was observed in group treated with ETH+PAS+ *Aloe vera* juice (R= 40.75gm ± 5.30 C= 59.25gm ± 5.30 and C/A = 19.75 gm ± 1.77) respectively.

The mean body weights were measured weekly (every 7 days) during the study. The mean body weight in normal control group is (292.5 gm ± 59.7). In experimental groups the minimum body weigh was found in animals treated with ETH+PAS+ *Aloe vera* juice (270.7 gm ± 51.0), Where as maximum body weight was recorded in animals treated with ETH+ *Aloe vera* juice (314.0 gm ±49.1). The mean body weights present in animals treated with *Aloe vera* juice only was (276.8 gm ± 59.5).

The mean relative weight of liver in control group was calculated and was found to be (9.737 gm ± 1.622). In experimental rats the minimum weight of liver was recorded in group treated with PAS+ *Aloe vera* juice (9.607gm ±1.843), whereas the maximum weight of liver was noted in rats treated with ETH (13.92 gm ± 3.667). The mean relative weight of liver in animals treated with *Aloe vera* juice only was estimated as (10.580 gm3.783).

Table-2: Showing the mean concentration of Liver Serum Biochemistry of Effect of Aloe vera joice and drugs Ethionamide and Para amino salicylic acid, on Sprague- dawley rats

The mean concentration of total Serum Bilirubin (T. Bili) estimated in normal control group was (0.42 mg/dL ±0.13). The minimum mean concentration of total serum bilirubin was found in animals treated PAS + *Aloe Vera* juice (0. 46 mg/dL ± 0.11), whereas the maximum concentration was noted in animals treated with ETH, PAS, ETH +PAS and ETH +PAS + *Aloe vera* juice (0.53 mg/dL ± 0.06). In animals treated only with *Aloe vera* juice, the mean concentration of total serum bilirubin was estimated as (0.51 mg/dL ±0.10).

The mean concentration of Serum Acid Phosphatase (ACP) found in normal control group was (0.06 IU/L ± 0.007). In case of treated groups, the minimum mean serum acid phosphatase was noted in group treated with PAS + *Aloe vera* Juice (0.05 IU/L ± 0.007). The maximum level of mean serum acid phosphatase was found in group treated with ETH + PAS (0.07 IU/L L ± 0.007). In group treated only with *Aloe vera* Juice, the level of mean serum acid phosphatase found to be (0.04 IU/L L ± 0.007).

The mean total Serum Alkaline Phosphatase (ALP) was estimated in normal control group was (362.40 IU / L± 107.74). The minimum mean total serum alkaline phosphatase was found in group treated with ETH + *Aloe vera* juice (307.52 IU / L ± 109.42). The maximum mean total serum alkaline phosphatase was found in group treated with ETH + PAS (410.95 IU / L ± 127.47), whereas the group of animals treated only with *Aloe vera* juice, the mean total serum alkaline phosphatase was estimated as (354.28 IU / L ± 122.57).

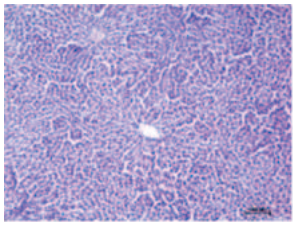
The mean total Serum Glutamic Pyruvic Transaminase (SGPT or ALT)) was recorded in normal control group was (67.89 IU / L ± 23.48). In case of treated groups, the minimum mean total serum glutamic pyruvic transaminase was found in animals treated with PAS + *Aloe vera* juice (63.54 IU / L ± 11.96), whereas the maximum mean total serum glutamic pyruvic transaminase was found in animals treated with ETH + PAS (97.24 IU / L ± 13.32). In case of animals treated only with *Aloe vera* Juice, the level of mean total serum glutamic pyruvic transaminase was estimated as (68.09 IU / L ± 7.83).

The mean total Serum Glutamic Oxaloacetic Transaminase (SGOT or AST) was recorded in normal control group was (188.26 IU / L ± 64.61). In case of treated groups, the minimum mean total serum glutamic oxaloacetic transaminase was found in animals treated with PAS + *Aloe vera* juice (180.68 IU / L ± 66.37), whereas the maximum mean total serum glutamic oxaloacetic transaminase was found in animals treated with ETH + PAS (239.73 IU / L ±77.41). In case of animals treated only with *Aloe vera* Juice, the level of mean total serum glutamic oxaloacetic transaminase was estimated as (179.54IU / L ± 7.83).

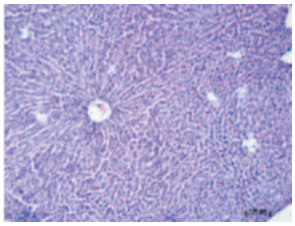
The mean concentration of HDL Cholesterol found in normal control group was (10.63 mg/dL ± 8.62). In case of treated groups, the minimum mean HDL Cholesterol was noted in group treated with PAS (8.66 mg/dL ± 2.53). The maximum level of mean HDL Cholesterol was found in group treated with PAS+ *Aloe vera* juice (36. 37 mg/dL ± 5.68). In group treated only with *Aloe vera* juice, the level of mean HDL Cholesterol found to be (23.75 mg/dL ± 8.88).

The mean total Triglycerid (TGL) was estimated in normal control group was (99.33mg/dL ± 16.70). The minimum mean total triglycerid was found in group treated with ETH (107.56 mg/dL ± ± 18.96). The maximum mean total triglycerid was found in group treated with ETH + PAS (138.06 mg/dL ± 22.51), whereas the group of animals treated only with *Aloe vera* juice, the mean total triglycerid was estimated as (115.27mg/dL ± 18.86).

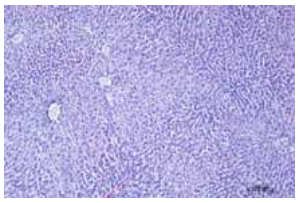
The mean Total Cholesterol (TC) was estimated in normal control group was (72.19 mg/dL ±10.36). The minimum mean total cholesterol was found in group treated with ETH+PAS (61.59 mg/dL ± 13.15). The maximum mean total cholesterol was found in group treated with PAS + *Aloe vera* juice (87.43mg/dL ± 18.17), whereas the group of animals treated only with *Aloe vera* juice, the mean total cholesterol was estimated as (70.09 mg/dL ± 7.75).



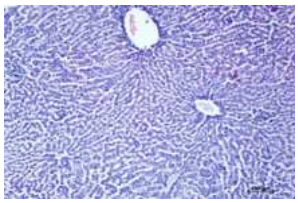
A-NC : Animals fed with rat pellets and ordinary water:



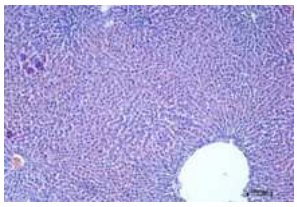
B- Animals treated with ETH 132 mg/kg



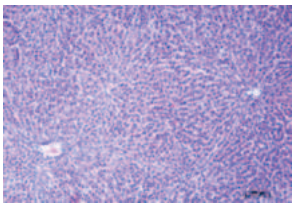
C- Animals treated with PAS 400mg/kg



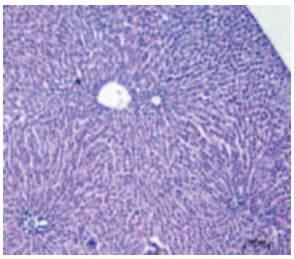
D– Animals treated with ETH + PAS 90 mg/kg



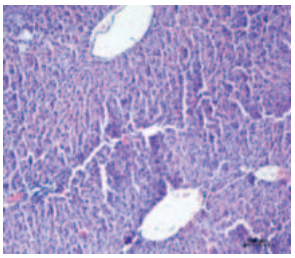
E- Animal treated with ETH+Aloe vera juice 90mg/kg



F- Animal treated with PAS +Aloe vera juice 90mg/kg



G- Animal treated with ETH+ PAS+ Aloe vera juice 90mg/kg



G- Animal treated with ETH+ PAS+ Aloe vera juice H- Animal treated with Only Aloe vera juice 90mg/kg

Photograph 1 (A- H): Showing Effect of Aloe vera joice and drugs

Ethionamide and Para amino salicylic acid, on the histological alteration in the Liver of Sprague- dawley rats

Group- A: NC (Normal Control):
The liver showing normal hepatic parenchyma with normal histomorphology of hepatocytes with intact cellular features. The hepatocytes were arranged in hepatic strands around the central vein. The hepatocytes appeared polygonal to round in shape with presence of round nucleus and intact cell borders. No any metabolic or pathological cellular lesions were noted in the liver.

Group- B: Animals treated with ETH:
The liver showing mild degenerative changes in the hepatocytes and around the central vein. The multifocal areas of hepatic vessels showed mild degeneration in cellular swelling of the hepatocytes. The enlarged nucleuses with granular cytoplasmic changes were observed. The minimal vacuolar changes in few hepatocytes were also noted. The congested blood vessels with multifocal areas and moderate hemorrhages in the hepatic parenchyma were observed.

Group- C: Animals treated with PAS:
The mild degenerative changes in the hepatocytes were observed around the central vein. The multifocal areas with mild hepatic degeneration of the cellular swelling in hepatocytes were noted. The enlarged nucleuses with granular cytoplasmic changes were also observed. The minimal vacuolar changes in few hepatocytes were also noted. The congested blood vessel with multifocal areas of hemorrhages in the hepatic parenchyma was also observed.

Group- D: Animals treated with ETH + PAS:
The mild to moderate degenerative changes in hepatocytes were observed around central vein. Multifocal areas of hepatic degeneration with cellular swelling of hepatocytes were noted with enlarged nucleus and granular cytoplasmic changes were noted. Multifocal vacuolar changes in few of the hepatocytes were noted. The congested blood vessels with multifocal areas of hemorrhages in the hepatic parenchyma observed. Focal infiltration of mononuclear cells was noted in hepatic tissue with focal congestion of vascular tissue.

Group- E: Animal treated with ETH+ *Aloe vera* juice:
The focal congested vascular tissue in hepatic parenchyma noted. The focal areas showing fatty infiltration with the presence of fatty droplets in the cytoplasm of hepatocytes was observed. The focal degenerative changes in hepatocytes with granular cytoplasm and focal cellular swelling of hepatocytes were also noted.

Group- F: Animal treated with PAS +*Aloe vera* juice:
The focal area with congested vascular tissue in hepatic parenchyma noted. The Focal areas of fatty infiltration with presence of fatty droplets in the cytoplasm of hepatocytes were observed. The focal degenerative changes in hepatocytes with granular cytoplasm and focal cellular swelling of hepatocytes were observed.

Group- G: Animal treated with ETH+ PAS+ *Aloe vera* juice:
The normal hepatic parenchymas with normal histomorphology of hepatocytes with intact cellular features were observed. The hepatocytes were arranged in hepatic strands around central vein. The hepatocytes appeared polygonal to round in shape with the presence of round nucleus and intact cell borders. There was no any noticeable metabolic or pathological cellular lesions were observed in the liver.

Group-H: Animal treated with *Aloe vera* juice only:
The normal hepatic parenchymas with normal histomorphology of hepatocytes with intact cellular features were observed. The hepatocytes were arranged in hepatic strands around central vein. The hepatocytes appeared polygonal to round in shape with the presence of round nucleus and intact cell borders. There was no any noticeable metabolic or pathological cellular lesions were observed in the liver.

The dose of acetaminophen administered to albino rats induced hepatotoxicity by elevating the level of ALT, ALP, and AST in the serum. The levels of these serum enzymes were significantly well decreased by *Hedera helix* leaf extract, which provide hepatoprotection as well as antioxidant characteristics against acetaminophen-induced liver damage [30]. The previous studies were done on *Aloe vera* extract reported that, ALP , AST and bilirubin are responsive biomarker acted directly on liver tissues to cure damage tissues and liver toxicity [31].The study demonstrated by [32] on the effect of *Rhaphiostylis beninensis* root extract against toxicity induced by(CCl4) on male albino rats. They found Liver enzymes AST, ALT, ALP and LDH are the indicators showed liver inflammation and necrosis, and significant increases in activities in animals treated with CCl4 alone compared to other groups. Whereas, the animals treated with *Rhaphiostylis beninensis* root extract showed lowering the level of AST, ALT, ALP and LDH and showed hepatoprotection against toxicity induced by (CCl4). They found the that the *Rhaphiostylis beninensis* root extract showed improvement by enhancing liver function and cellular integrity as well as the antioxidative status of the liver of male albino rats. The ETH and PAS induced hepatoxicity in *Sprague-dawley* rats by increasing the levels of serum enzymes ACP, ALP, AST, ALT, and Bilirubin. However the test animals treated with ethanolic extract of *Piper nigrum* seeds showed ameliorated the toxic effect of the drugs [33]. The enzymes showing significant increase in the levels of AST, ALT, ALP, and ACP, bilirubin in male *Wistar rats* treated with isoniazid and rifampicin drug. However, these serum enzymes were found decreased in rat treated with *Aloe vera* juice [34]. In another study *Aloe vera* polysaccharides administration in combination with aflatoxins B1 in albino rats showed reversal hepatotoxicity by lowering the level of serum ALT and AST significantly near to normal as compared to rats treated with aflatoxins B1 [35].The study carried out by[36] on *Aloe vera* attenuated liver injury in mice with acetaminophen-induced hepatitis found the increased levels of serum transaminase, SGOT and SGPT which indicates liver damage. They found the increased level of serum SGOT and SGPT were significantly increased in APAP group when compared with control group and significantly decreased in *Aloe vera* treated group compared with APAP group. The results of this investigation showed that *Aloe vera* juice administration significantly reduced serum ALT, AST, TC, and TG, which is consistent with the finding of [37]. The rabbits treated with malathion remarkable increased the level of serum enzyme activity of LDH, ASAT, ALAT and ALP. Whereas the rabbits administered the *Aloe vera* juice in protective and therapeutic group found to be lowering the elevated levels of enzymes to nearly normal by protecting the liver [38]. The study carried out by the [39], and revealed the hepatotoxicity induced rabbits showed significantly increased levels of ALT, AST and ALP. The *Aloe vera* juice administered in treat group found to be effective by reducing the levels enzymes. Bilirubin is usually conjugated with glucuronic acid in the liver in a reaction catalyzed by bilirubin-UDP-glucuronyltransferase which solubilizes it and is subsequently excreted in bile [40]. Furthermore, administration of the extract (*Aloe vera*) for 30 days in anti-tuberculosis drugs induces hepatotoxicity, leading to restoration of liver function enzymes. The results also support that *Aloe vera* juice can be used in combination with multiple medications for patients with chronic disease to protect their liver function as dietary bioactive substances help combat chronic metabolic dysfunction [41]. The administration of paracetamol in albino rats significantly increased the level of serum AST, ALT, ALP, bilirubin, cholesterol and LDH by inducing the development of hepatotoxicity as compared to the control group. However, the rats treated with Paracetamol and *A. niebuhriana* latex extract caused significant decreased in the enzyme level by restoring the liver to its normal function [42]. The adverse effect of anti-Tb drugs medications reduce the treatment effectiveness by compromising treatment regimens, but it also cause serious and adverse effect on liver by inducing hepatotoxicity [43,44]. Rifampicin and isoniazid have been reported to induce hepatotoxicity based on the presence of increased serum AST, ALT, ALP and total bilirubin levels, focal hepatocytic necrosis and portal triademia [45]. Rifampicin and isoniazid, alone or combined, are still widely used in most antitubercular chemotherapeutic regimens.

However, these drugs are considered hepatotoxic agents [46,47]. Oxidative stress is one of the mechanisms that play a central role in the pathogenesis of hepatitis induced by antitubercular drugs (isoniazid and rifampicin). Administration of isoniazid and rifampicin, individually and in combination, led to a significant disturbance in liver function as measured by changes in serum enzymes (AST, ALT, ALP and ACP) as well as bilirubin, total proline, total albumin and total globulin and liver histopathology [48]. Whenever the liver cell membrane is damage, it is well known that the enzyme aminotransferase is very sensitive indicator of liver cell injury and is released into the blood in increasing amount [49]. The experiments on animal models suggested that the administrations of antitubercular drugs increase the level of ALT, AST and ALP in serum, affecting the hepatocellular membrane and its organelles [50, 51]. The increased level of bilirubin helps to

determine the integraty of liver. The hyper activity of hepatocytes leads to hyper billirubinaemia [52]. Bilirubin is a useful indicator of excretory function of liver. The abnormal increase in the bilirubin indicates hepatobiliary disease with severe disturbances in the hepatocellular structure which leads to disease condition [53]. It is reported that sub acute or chronic treatment of isoniazid drug induce hepatotoxicity in man [54,55] and guinea pigs [56], resulting in the increased levels of serum transaminases and phosphatase activities. The treatment regimes of isoniazid induced hepatitis are associated with ballooning degeneration, focal necrosis on the hepatocytes with minimal cholestasis [54].

In our present study, In the case of mean food consumption rate, mean body weights, and mean relative weights of liver was calculated in normal control and the rats treated groups. From the above results it was found that, statically no significant difference ($p<0.001$ was noted in mean rate of food consumption, mean body weights and mean relative weight of liver, when compared with the normal control groups. The mean concentration of bilirubin was found increased in rats ETH, PAS, ETH +PAS and ETH +PAS + *Aloe vera*, as compared to control group and rats treated with *Aloe vera* juice. The level of bilirubin was found increased but the statistically no significant difference ($p<0.001$) was noted as compared with normal control group. The mean concentration of ACP was found elevated in rats treated with ETH + PAS, whereas the ACP was found decreased in rats treated with PAS + *Aloe vera* Juice as compared to *Aloe vera* treated and control group. In case of ACP there was no statistically significant ($p<0.001$) change observed in group treated with ETH + PAS when compared to *Aloe vera* Juice treated and normal control group. The levels of ALP were found to be significantly increased in group treated with ETH + PAS ($p<0.05$) when compared to normal control animals. These levels were found to be significantly decreased in ETH+ *Aloe vera* juice group ($p<0.05$).In ETH + PAS group, although the ALP levels were increased as compared to animals treated only with *Aloe vera* juice also but the difference was not statistically significant. In case of ALT (SGPT) levels were significantly increased in ETH + PAS groups ($p<0.001$) as compared to normal animals. Although the ALT (SGPT) levels were found to be decreased in PAS +*Aloe vera* juice treated groups, as compared to the normal control and animals treated only with *Aloe vera* juice the difference was statistically significant ($p<0.05$). Thus it proves the hepatotoxicity. In case of AST (SGOT) levels were significantly increased in ETH + PAS groups ($p<0.05$) as compared to normal animals. Although the AST (SGOT) levels were found significantly decreased in PAS + *Aloe vera* juice treated groups, as compared to the normal control and animals treated only with *Aloe vera* juice the difference was statistically significant ($p<0.05$).In case of HDL Cholesterol levels were significantly increased in group treated with PAS+ *Aloe vera* juice groups ($p<0.05$) as compared to normal animals. Although the HDL levels were found significantly decreased in group treated with PAS, when compared to the normal control and animals treated only with *Aloe vera* juice the difference was statistically significant ($p<0.05$).In case of Triglycerid (TGL) levels were significantly increased in in group treated with ETH + PAS as compared to normal animals. The minimum mean total TGL was found in group treated with ETH. It was found that the total TGL found no significantly decreased in normal animals and the animal treated only with *Aloe vera* juice the difference was statistically not significant($p<0.001$).In case of total cholesterol (TC) the maximum level was found in group treated with PAS + *Aloe vera* juice as compared to normal control animals. Whereas minimum meanTC was found in group treated with ETH+PAS. There was no significant difference was noted in normal control and animals treated only with *Aloe vera* juice ($p<0.001$).

CONCLUSION:

From the present finding it was found that the *Sprague- dawley* rats treated with ETH and PAS showed an increased levels of bilirubin, ACP, ALP, ALT, AST, HDL, and lowering the levels of TGL, total cholesterol. However, the rats orally administered of Aloe vera juice in combination with the ETH and PAS or independently *Aloe vera* juice found to be effective in lowering the elevated activities of bilirubin, ACP, ALP, ALT, AST, HDL to approximate normal level in ETH and PAS treated rats and by increasing the level of TGL. From the histopathalogical study it was also found that ETH and PAS could induce hepatitis through increases in oxidative stress, liver injury, and liver histopathology. The current research implies the functional potential of *Aloe vera* juice in hepatotoxicity induced rats. The *Aloe vera* juice has anti –inflammatory, and antioxidant property that could prevent these adverse events and might be used as an adjunctive therapy for ETH and PAS –induce hapatoxicity. The *Aloe vera* has devised functional properties that could be useful for reducing multiple clinical ailments related to liver disease or drug induced hepatotoxicity. However, further investigations are required and should be conducted including biomarkers of gene expressions and metabolomics in order to find exactly the mechanism of action of *Aloe vera* juice against ETH and PAS induce hapatoxicity.

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