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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF AMALGAMATION OF KUTKUTI (TRIDAX PROCUMBENS) AND GINGER JUICE AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS.

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Abstract
The hepatoprotective activity of amalgamation of tridax procumbens and ginger juice was evaluated against paracetamol induced hepatotoxicity in rats. The albino wistar rats (n = 30) were randomly divided into 6 groups (n = 5). Group I (Normal control) did not receive any treatment. Group II (Toxic control) animals were administered with paracetamol (2.75g/kg p.o.) on 6th and 7th day. Group III rats pretreated with silymarin at a dose of 50 mg/kg b. w. /day for 7 days and were induced with Paracetamol at dose 2.75g/kg p.o. on 6th and 7th day. Group IV rats pretreated with ethanolic extract of tridax procumbens at dose 200mg/kg for 7 days and were induced with paracetamol at dose 2.75g/kg p.o. on 6th and 7th day . Group V rats pretreated with ginger juice at dose 2ml/kg for 7 days and were induced with paracetamol at dose 2.75g/kg p.o. on 6th and 7th day. Group VI pretreated with ethanolic extract of tridax procumbens and ginger juice at 200 mg/kg and 2ml/kg dose b. w. /day for 7days and were induced with paracetamol at dose 2.75g/kg p.o. on 6th and 7th day. Liver function tests, triglyceride, MDA, glutathione and electrolyte profile were estimated using standard kits. Livers were quickly removed and fixed in 10% formalin and subjected to histopathological studies. In conclusion, the amalgamation of tridax procumbens and ginger juice can enhance antioxidant activity and ameliorate the paracetamol-induced hepatotoxicity. The results of this study strongly indicate that Amalgamation of tridax procumbens and ginger juice has got a potent hepatoprotective action against paracetamol induced hepatic damage in rats.

Keywords: Amalgamation of tridax procumbens and ginger juice: heptoprotection: paracetamol: rats: SGOT: SGPT: ALP

1. INTRODUCTION:

Liver is susceptible to drug-induced injury owing to its major role in drug metabolism and in detoxification and elimination of toxic substances. The liver is often affected by a multitude of environmental pollutants and drugs, all of which place a burden on this vital organ and can damage and weaken it, Several drugs such as antipyretics including acetaminophen (APAP), antiinflammatory drugs, antituberculosis drugs, antidepressants and anticancer drugs have potential hepatotoxicity. The incidence of drug-induced liver diseases is increasing day by day and unintentional drug toxicity is very common nowadays all over the world. The drug-induced liver diseases attribute more than 50% of acute liver failure[1]. Paracetamol's hepatotoxicity is caused by its reactive metabolite Nacetyl-p-benzoquinone imine (NAPQI), which causes glutathione (GSH) depletion. Paracetamol toxicity is

due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P450[2]. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity[3,4,5]. Hepatotoxicity is also termed as high serum glutamate pyruvate transminase (SGPT); high serum glutamate oxaloacetate transminase (SGOT). It is an elevation of triglycerides and depletion in the tissue GSH levels[6]. In spite of tremendous strides in modern medicine, the treatment of liver disorders is inadequate and many formulations containing herbal extracts are used for regeneration of hepatic cells and for protection of the liver against damage.[7] Hepatic damage is associated with distortion of its metabolic functions and it is still a major health problem.[8] The liver is the principal glandular organ in the body and has more functions than any other human organ. Liver is not only the secondlargest organ of the body but also the largest gland. Liver is weighing about 1.4 kg in grown person and is underline to the diaphragm occupying most of the right hypochondriac and a part of the epigastric region of abdominopelvic cavity[9]. Liver deals with many pathways which include energy provision ,nutrient supply, relevant to growth, fight against disease, and reproduction are corresponded with liver[10]. The liver has an inventory task of maintaining the body's metabolic homeostasis. The liver is expected to perform physiological functions as well as protects against the hazards of chemicals and harmful drugs. Unfortunately many synthetic drugs used in the treatment of liver diseases are inadequate and also cause serious side effects. In view of severe undesirable side effects of synthetic agents, there is growing interest in evaluating traditional herbal medicines that are claimed to possess hepatoprotective activity. A single drug cannot be effective for all types of severe liver diseases. Therefore, an effective formulation using indigenous medicinal plants has to be developed with proper pharmacological experiments and clinical trials.[11] Considering the above limitations, Amalgamation of tridax procumbens and ginger juice made up of with leaves of Tridax procumbens and rhizomes of Ginger was subjected to various assays. In order to evaluate its hepatoprotective effect, an amalgamation from the mixture of these herbs was tested against paracetamol-induced hepatotoxicity in albino rats.

The natural product with antioxidant properties showed hepatoprotective activity against paracetamol toxicity. Tridax procumbens (Asteraceae) has a long history of use in traditional medicine, particularly in India, where it is known as ghamra. Its leaves, flowers, and roots have been used to treat a wide range of health conditions, including fever, pain, skin diseases, respiratory infections, and digestive disorders[12]. A weed employed as indigenous medicine for a variety of ailments including jaundice[13].Several studies have been conducted on the medicinal properties of Tridax procumbens. It has been found to contain various bioactive compounds, such as flavonoids, alkaloids, terpenoids, and phenolic acids, which have antioxidant[14], Antihyperglycemic activity[15], Antiinflammatory activity. It has been extensively used as anticoagulant, antifungal and insect repellent; in bronchial catarrh, diarrhoea and dysentery[16]. Moreover, it possesses wound healing activity and promotes hair growth. Tridax procumbens is also dispensed as "Bhringrají", which is well known Ayurvedic medicine for liver disorders [17]. Ginger (Zingiber officinale) is an important medicinal plant belonging to the family Zingiberaceae. It is widely used in traditional medicine for the treatment of various diseases such as nausea, vomiting, and inflammatory disorders. Ginger contains various bioactive compounds such as gingerols, shogaols, and zingerone, which possess antioxidant and anti-inflammatory properties. Ginger has become a subject of interest because of its beneficial effects on human health. Ginger has a broad range of biological activities including anti-bacterial, anti-convulsant, analgesic, antiulcer, and anti-fungal[18,19]. There is also evidence that ginger may help in the treatment of cardiovascular disease due its anti-infiammatory, anti-platelet and hypolipidemic effects[20].

Therefore, the present investigation was under taken to evaluate hepatoprotective effect of amalgamation of Tridax procumbens and Ginger juice in paracetamol induced hepatotoxicty in rats which has not been earlier reported. It is our belief that this examination will take us another step forward in our quest to understand the mechanism of action of amalgamation of Tridax procumbens ang ginger in prevention and medicaments of liver related diseases.

2. MATERIALS AND METHODS:

2.1. Drugs& chemical

Paracetamol was acquired from cipla, Silymarin(silybon140,Micro lab) was purchased in a tablet form at strength 140 mg. All other chemicals and reagents used were of analytical grade and aquired from approved chemical suppliers. The serum glutamate oxaloacetate (SGOT), Serum glutamate pyruvate transminase (SGPT) and Serum alkaline phosphate (ALP)and Total bilirubin, total protein, Triglyceride, MDA, GSH and electrolyte profile were measured with the help of commercial kits.

2.2. Collection and extraction of plant material

The plant samples were collected at flowering stage from local region during April- May, (Kolki, Phaltan, Dist : satara). The plant was authenticated by Department of Botany, Y. C. Institute of Science, Satara. The leaves of Tridax procumbens were dried under shade and grinded in electrical grinder to coarse powder. Powdered leaves was prepared in 95% ethanol using maceration method extracted for 72 h, after which the resultant mixture was filtered and filtrate was stored in refrigerator for subsequent use. A known volume of this extract was concentrated at 45℃ in a water bath for complete dryness. Crude extract obtained was stored at 4℃ for further use. Fresh rhizome of ginger (Zingiber Officinale) were obtained from local market and confirmed botanically. Ginger juice was prepared using the method of Akhani et al. Fresh rhizome of ginger (1kg) were crushed and then squeezed in muslin cloth to obtain juice , which was stored in the refrigerator at 2-8℃ in a well closed glass container.

2.3. Experimental animals

The complete experiment was carried out using 30 wistar albino rats of weighing 150 - 200g.
The study protocol was approved by Institutional Animal Ethics Committee (IAEC) ,YTC, YSPM, Satara. The animals were aquired from registered breeder and familiarized in the quarantine area for one week. Animals were housed in clean polypropylene cages in a controlled room temperature 22℃ ± 2℃, relative humidity of 50 ± 15% and 12 hr dark/ 12 hr light cycle at our Institution's animal house and allowed to acclimmatized for one week. The animals were fed with water and standard pellet diet *ad libitum*. Animals were maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animals Guidelines.

2.4. Induction of hepatotoxicity

On 6^{th} and 7^{th} day, all the experimental animals excepts control group ,were administered Orally with Paracetamol at a dose of 2.75g/kg dissolved in distilled water.

2.5. Experimental design

A total 30 wistar albino rats were randomly divided into 6 groups containing 5 animals in each group.
Group I (Normal control): did not receive any treatment.
Group II (Toxic control): animals were administered with 2.75g/kg b. w. per oral dose of paracetamol on 6^{th} and 7^{th} day
Group III [Standard] rats pretreated with silymarin at a dose of 50 mg/kg b. w. /day for 7 days and were induced with Paracetamol at dose 2.75g/kg on 6^{th} and 7^{th} day.
Group IV [Test I] rats pretreated with ethanolic extract of tridax procumbens at dose 200mg/kg for 7 days and were induced with paracetamol at dose 2.75g/kg on 6^{th} and 7^{th} day .
Group V [Test II] rats pretreated with ginger juice at dose 2ml/kg for 7 days and were induced with paracetamol at dose 2.75g/kg on 6^{th} and 7^{th} day.
Group VI [Test III] pretreated with ethanolic extract of tridax procumbens and ginger juice at 200 mg/kg and 2ml/kg dose b. w. /day for 7days and were induced with paracetamol at dose 2.75g/kg on 6^{th} and 7^{th} day.

2.6. Acute oral toxicity in rats:
Acute toxicity study of the extract was done according to acute toxic classic method (OECD guideline 423, 2006) using albino female rat to determine the safe dose. The animals were kept fasting for overnight with sufficient water. . Initially, a single dose of tridax procumbens (2000 mg/kg) and ginger juice (20ml/kg) was given to a rat and was closely observed for mortality, general behavior and signs of discomfort for 7 days.

27. Collection of blood sample:
On 8th day blood was collected through retro orbital puncture and analyzed for various biochemical parameters. Blood was allowed to clot at room temperature for 30 min, subjected to centrifugation (3000 rpm for 15 min.) and estimation of biochemical parameters.

2.8. Determination of wet liver weight:
Animals were sacrificed and livers were isolated and washed with saline and weights determined by using an electronic balance. The liver weight were expressed with respect to its body weight i.e. gm/100gm.

2.9. Determination of Wet liver Volume:
After recording the weight, all the livers were dropped individual in a measuring cylinder containing a fixed volume of distilled water or saline and the volume displaced was recorded.

2.10. Liver function test parameters
At the end of experimental period, animals were kept fasted over night and anaesthesized with chloroform. Blood samples were collected serially by retro orbital puncture. The blood was allowed to clot for 30 min at room temperature then serum was separated by centrifugation. Liver function tests like serum glutamic oxaloacetic transaminase (AST), serum glutamic pyruvate transaminase (ALT), and alkaline phosphate (ALP) levels of serum, total bilirubin, total protein, triglyceride level were determined using standard kits. [21-24)

2.11. Determination of MDA and hepatic GSH
2.11.1. Tissue MDA level
The MDA contents of the homogenates were analyzed in compliance with the method reported by Gutteridge and Wilkins. [25] The assay mix was prepared by combining 1 mL of glacial acetic acid, 1 mL of 1% thiobarbituric acid solution and 0.2 mL of sample. After zeroing the spectrophotometer with a blank containing 0.2 mL of distilled water in the place of the sample, they were read at 532 nm.

2.11.2. Tissue GSH level
The sample was deproteinised with 5-sulfosalicylic acid. The GSH in liver tissue was measured by using kinetic assay in which catalytic amounts of GSH (nmol) caused continuous reduction of 5,5'dithiobis (2-nitrobenzoic acid) to 5-thio-2- acid. The yellow product 5-thio-2nitrobenzoicacid was measured at 412 nm. The GSH level of liver wasexpressed in nmol/mg.

2.12. Determination of electrolytes profiles
The sodium, potassium, chloride contents of the serum were assayed according to the kits manufacturer's instructions.

2.13.Histopathology of liver
animals were anaesthetized in chloroform using a desiccator. The liver was quickly separated and fix in 10% buffered formalin. The tissues were sectioned at a thickness of 5 μm, bathed in paraffin, and stained with hematoxylin and eosin (H&E). Photomicrographs were taken, and the slices were viewed under light microscope.

2.14. Statistical analysis

The statistical analysis was carried out with Graphpad prism 5.0 software. The data was statistically analyzed using one-way ANOVA followed by Tukey's multiple comparison tests and $p< 0.05$ was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1. Acute oral toxcity:

The acute oral toxicity (LD_{50}) results demonstrate that single dose treatment with tridax procumbens (2000mg/kg)and ginger juice (20ml/kg) did not cause any behavioral change or death of rat.

3.2. Body weight

The body weight, the behaviour and physical activity of the animals were monitored daily. The drastic drop of body weight was found in experiment group after 24 h of last dose of paracetamol.
However, the body weight regained back to normal in all subgroups at the end of the experiment.

Table.1. Effect of amalgamation of Tridax procumbens 200mg/kg and ginger juice 2ml/kg on body weight

Groups	Treatment	Initial weight (gm)	Final weight (gm)
Control	Distilled water	203±6.3	211±8.1
Toxicant control	Paracetamol(2.75g/kgb.w.)	192±4.9	180±5.3
Standard	Silymarin (50mg/kg b.w.)	182±9.7	188 ±7.1
Test I	(Tridax procumbens 200mg/kg b.w.)	181±6.2	185 ±3.1
Test II	(Ginger juice 2ml/kg b.w.)	177±8.1	180 ±5.2
Test III	(Tridax procumbens200mg/kg + ginger juice2ml/kg)	190±8.7	195 ±8.4

Values are mean ± standard error of the mean (SEM), n=5 animals, per group.

3.3. Liver weight and Liver volume

Paracetamol treatment in rats showed the hepatic damage which was evident by increase in the liver weight and volume. When treated with standard (Silymarin) and amalgamation of tridax procumbens and ginger juice showed good reduction in weight and volume of liver.

Table2: Effect of amalgamation of Tridax procumbens 200mg/kg and ginger juice 2ml/kg on Liver weight and volume of Paracetamol induced hepatotoxicity in experimental animals.

Experimental group	Liver weight gm/100gm	Liver volume ml/100gm
Normal control	4.03±0.31	6.23 ±0.24
Toxic control	6.52 ±0.57	9.10 ±0.01
Standard	4.51 ±0.21	5.64 ±0.02
Test I	4.24 ±0.20	8.81 ±0.25
Test II	4.22 ±0.90	7.42 ±0.31
Test III	4.42 ±0.10	5.93 ±0.12

Values are mean ± standard error of the mean (SEM), n=5 animals, per group.

3.4. Effect on Biochemical parameter

The hepatoprotective effect of amalgamation of ethanolic extract of TP and ginger juice on liver function test in paracetamol intoxicated rats is presented in Table 3. Intoxication of rats with paracetamol resulted in significant elevation in AST, ALT and ALP relative to the normal control group ($P<0.05$). Besides, rats pretreated with amalgamation of ethanolic extract of tridax procumbens and ginger juice and the reference drug, silymarin significantly reduced AST, ALT, and ALP respectively when compared to the toxic control group ($P<0.05$). The paracetamol intoxicated rats

had significantly elevated total bilirubin and triglyceride as well as significantly decreased total protein and albumin compared to the normal control group (P<0.05). However, administration of amalgamation of ethanolic extract of TP and ginger juice and also silymarin showed significantly decrease in bilirubin and triglyceride, as well as significantly elevated total protein and albumin compared to the toxic control group with all P<0.05 (Table 3).

Table 3. Effect of amalgamation of tridax procumbens and ginger juice on liver function test levels in paracetamol induced hepatotoxicity in rats:

Group	SGOT(AST) (IU/L)	SGPT(ALT) (IU/L)	ALP (IU/L)	Triglyceride (mg/dl)	Bilirubin (mg/dl)	Protein g/dl	Albumin g/dl
Normal control	68.80±12.04	51.70± 8.02	77.01±13.03	133.51±16.1	0.42±0.08	6.72± 0.51	4.2±0.11
Toxic control	255.4±15.08	128.02±11.9	294.45±14.04	254.01±15.02	0.81±0.14	3.18±7.11	2.5±0.18
Standard	88.7±11.10	55.91±5.52	182.02±8.08	125.30±17.2	0.52±0.02	6.91±0.69	4.4±0.13
Test I	209.4±13.11	118.07±15.4	255.05±13.04	185.32±16.2	0.61±0.06	4.1±0.41	3.0±0.14
Test II	189.3±10.53	122.04±14.1	230.30 ±1.78	160.02±11.4	1.68±0.16	4.4 ±0.42	3.3.0±0.12
Test III	96.04±12.41	84.4±9.10	190.01±3.8	130.54±10.8	0.54±0.15	6.2 ±0.51	4.1±0.18

Values are mean ± standard error of the mean (SEM), n=5 animals, per group. Values in the same column with different superscript symbols differ significantly at P<0.05. Values significantly differ from normal control (p<0.05). Values significantly different from paracetamol(toxic control). Normal control: Distilled water, Toxic control: Paracetamol, Standard: Silymarin, Test I: Tridax procumbens, Test II: Ginger juice, Test III: Amalgamation of tridax procumbens and ginger juice

3.5. Tissue MDA and GSH level

The effects of Amalgamation of ethanolic extract of tridax procumbens and Ginger juice on Tssue MDA and GSH level in paracetamol treated rats is shown in Table. 4. The hepatic MDA concentration of Toxic control was significantly (P<0.05) higher. The amalgamation of tridax procumbens and ginger juice showed significant reduction in MDA level compared to paracetamol group.

The hepatic glutathione level was significantly (P<0.05) lower in toxic control group as compare to normal control. The amalgamation of Tridax procumbens and ginger juice and silymarin significantly restored the GSH level as compare to toxic group.

Table 4. Effects of the amalgamation of tridax procumbens and ginger juice on Tissue MDA and hepatic GSH level

Groups	**MDA(nmol/mg)**	**GSH (nmol/ mg)**
Normal control	3.0±0.440	3.5±0.023
Toxic control	4.5±0.65	0.8±0.025
Standard	2.31±0.038	3.2±0.025
Test I	3.804±0.338	1.9±0.048
Test II	3.74±0.166	2.5±0.046
Test III	2.93±0.197	2.90±0.03

Values are mean ± standard error of the mean (SEM), n=5 animals, per group. Values in the same column with different superscript symbols differ significantly at P<.05. Values significantly differ from normal control (p<0.05). Values significantly different from paracetamol(toxic control). Normal control: Distilled water, Toxic control: Paracetamol, Stanard: Silymarin, Test I: Tridax procumbens, Test II: Ginger juice, Test III: Amalgamation of tridax procumbens and ginger juice

3.6. Determination of electrolytes profiles

Paracetamol intoxication of Wistar rats resulted in significant elevation of Na^+ and depression of Cl^- and K^+, compared to the normal control group (all P < 0.05). Nevertheless, hepatoprotective activity

following pretreatment with amalgamation of ethanolic extract of TP and silymarin presented significant depression of Na^+ and significant elevation of Cl^- and K^+ compared to toxic control group with all $P < 0.05$ (Table 5).

Table 5. Effects of the extracts on the hepatic electrolytes profiles of paracetamol treated rats

Treatment	Chloride (mmol/L)	Potassium (mmol/L)	Sodium (mmol/L)
Normal control	103.0±0.14	47.9±1.02	140.0±1.8
Toxic control	90.0±0.14	32.20±1.01	158.0±1.84
Standard	100.0±0.19	44.7±0.13	145±2.72
Test I	95.0±0.16	57.12±0.28	153.0±1.80
Test II	97.0±0.22	43.1±0.49	127±1.63
Test III	99.0±0.34†	44.2±0.23	143±1.517

Values are mean ± standard error of the mean (SEM), n=5 animals, per group. Values in the same column with different superscript symbols differ significantly at P<.05. Values significantly differ from normal control ($p<0.05$) Values significantly different from paracetamol(toxic control), Normal control: Distilled water, Toxic control: Paracetamol, Standard: Silymarin, Test I: Tridax procumbens, Test II: Ginger juice, Test III: Amalgamation of tridax procumbens and ginger juice

3.7. Histopathological studies

The histopathological evaluation of Ethanol toxicity in all the groups was examined and shown in Figure. The histopathological examination of normal control group showed normal structure and architecture Fig(a). Paracetamol intoxicated rats showed cellular degeneration. necrosis and inflammatory changes associated with fatty changes were noted Fig(b). The section of liver treated with Silymarin 50 mg/kg body weight showed almost normal architecture of the liver compare to control animal Fig(c). The section of liver treated with ethanolic extract of TP 200 mg/kg showed less centrilobular necrosis. Central vein and sinusoid congestion were observed. Mild degree of inflammation and fatty changes were noted Fig(d). The section of liver treated with ginger juice 2ml/kg showed less centrilobular necrosis. Central vein and sinusoid congestion were not observed. Mild degree of inflammation and fatty changes were noted Fig(e). Treatment with combination of ethanolic extract of tridax procumbens and ginger juice showed a pathological protection to liver. The section of liver showed normal architecture of liver, no degeneration, and found to reduce inflammation, centrilobular necrosis Fig(f). Liver section of this group shows normal hepatocytes with significant reduction in areas of necrosis when compared to toxic group. These changes show protective effect of the drug against hepatic damage induced by paracetamol

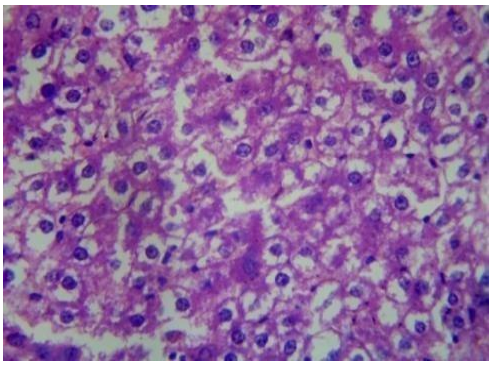

Fig.1 Section of the liver tissue of control rat

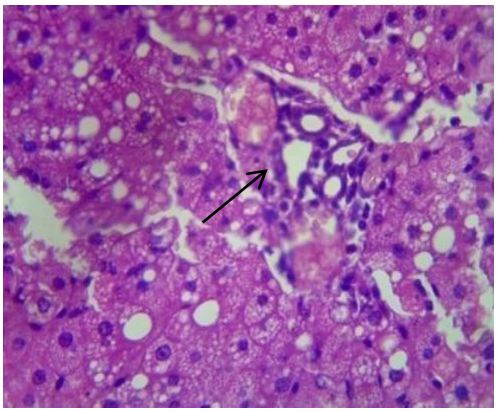

Fig.2. Section of liver tissue of paracetamol intoxicated rat

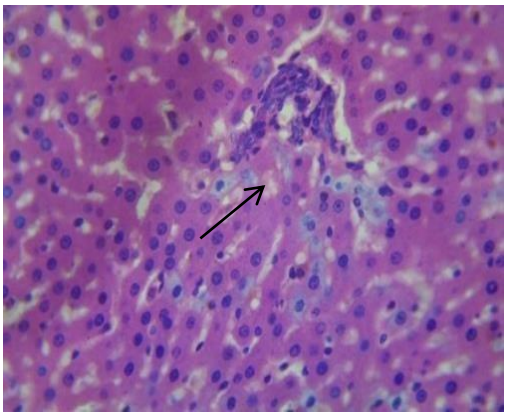

Fig.3. Section of liver tissue of rat treated with Silymarin 50 mg/kg

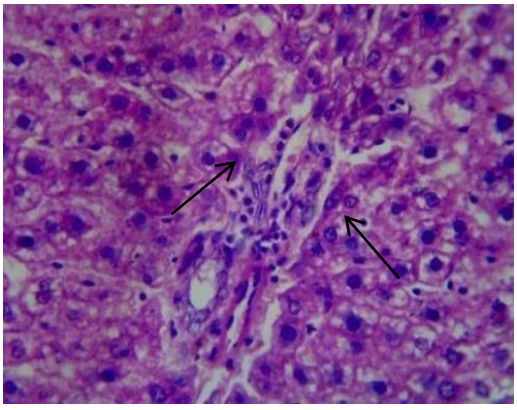

Fig.4. Section of liver tissue of rat treated with ethanolic extract of tridax procumben

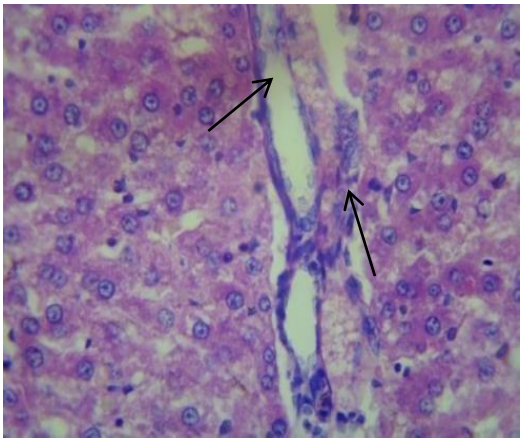

Fig.5. Section of liver tissue of rat treated with ginger juice.

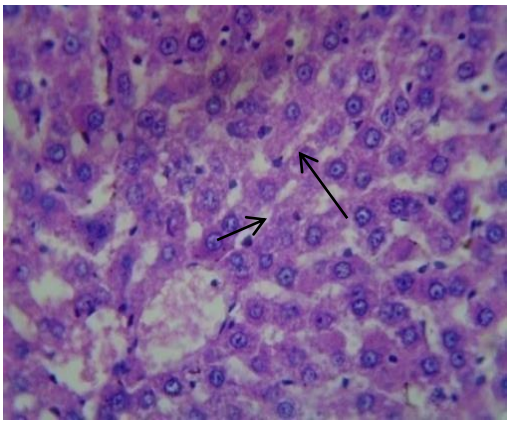

Fig.6. Section of liver tissue of rat treated with amalgamation of ethanolic extract of tridax procumbens and ginger juice.

4. CONCLUSION

In conclusion, the amalgamation of Tridax Procumbens and ginger juice offered more protection in preventing or treating paracetamol induced hepatotoxity. This could be due to principle of synergy, which suggests that the combination of two or more substances can results in greater effectiveness than any of the individual substances alone. The possible mechanism responsible for the protection of paracetamol-induced hepatotoxicity by combination of Tridax procumbens and ginger juice may be that it could act as a free radical scavenger intercepting the radicals involved in paracetamol metabolism by microsomal enzymes. The maximum effect was seen with combination of tridax procumbens and ginger juice than the alone in hepatoprotective study. Overall, amalgamation of Tridax procumbens and ginger may provide a more potent and safer approach to treating paracetamol induced hepatotoxicity in rats.

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