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Hepatoprotective Potential of *Hylocereus Polyrhizus* (Dragon Fruit) on Carbon Tetrachloride Induced Hepatic Damages in Albino Wistar Rats

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Abstract

This study was conducted to evaluate and determine the pre and post effect of *Hylocereus polyrhizus* on carbon tetrachloride (CCl_4) induced hepatic damages in albino wistar rats. This study applied the maceration method to produce ethanolic extract. Approximately one hundred grams of sample was macerated separately in 200mL of 95% ethanol in a well stoppered Erlenmeyer flask for 72 hours. In obtaining the crude extracts, another sample was extracted using a blender. The filtrates collected from the ethanolic extract were evaporated by rotary evaporator. The ethanolic extracts were subjected to flame test to check the presence of alcohol. The Hepatoprotective activity was evaluated based on the changes of Serum Glutamic-Pyruvic Transaminase (SGPT) and Serum Glutamic-Oxaloacetic Transaminase (SGOT) values among the groups. There were observed significant decreases in SGPT and SGOT levels among rats administered with *Hylocereus polyrhizus* extracts. The *Hylocereus polyrhizus* crude and ethanolic extracts are both found effective as a protector of the liver against the induced carbon tetrachloride (CCl_4) hepatic damages. However, findings reveal that the hepatoprotective effect against Carbon Tetrachloride(CCL_4) induced hepatic damages is better with crude extract when compared with ethanolic extract.

Keywords: Hepatic damage; Hepatoprotective potential; Hylocereus Plolyrhizus; Carbon Tetrachloride Induced.

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1. Introduction

Weighing at around three (3) pounds, the liver is the second largest organ of the body[1]. It performs many essential functions related to digestion, metabolism, immunity, and storage of nutrients within the body.

The liver's main job is to filter the blood coming from the digestive tract, before passing it to the rest of the body. It detoxifies chemicals and metabolizes drugs[2]. These functions make the liver a vital organ without which the tissues of the body would quickly die from lack of energy and nutrients.

It has been recognized that liver diseases are among the serious ailments worldwide caused by toxic chemicals either by exposure or intake. Carbon Tetrachloride (CCl_4) for instance is a chemical included in the list of toxic chemicals that could be associated with serious liver injury[3]

Medications and diet may require alteration like protein restriction to limit the formation of ammonia. Chronic liver injury may also result in development of carcinoma (cancer) of the liver.

In view of the important role of the liver in the body system and the consequence of liver diseases coupled with expensive medicines, investigations for plants with certain favorable effect on liver disorders have been highly regarded in research. For instance, Phytochemical screening of some compounds from plant leaf extracts revealed that they contain medicinally bioactive compounds which justifies the use of plant species as traditional medicine for treatment of various diseases[4]. In one study, ethanolic extract of the root of P. Odoratissimus afforded significant protection against PCM induced hepatoxicity in rats[5]. It was also found out in one study that ethanolic extract of A. agallocha leaves(AAE) possesses hepatoprotective effect in SD rats[6]. Leaves of other medicinal plants were also tested and was found out to have hepatoprotective potential[7].

Cactaceae such as *Hylocereus polyrhizus* known as dragon fruit is among those plants that have gained cognizance in terms of its food and medicinal value. It has three (3) hybrid varieties depending on its skin and flesh: *Hylocereus undatus*, red-colored skinned with white flesh; *Hylocereus polyrhizus*, red skinned with red flesh and *Selinecereus Megalanthus*, yellow-colored skinned with white flesh. Different types have different nutritional values[8]. Two of the varieties were found out to contain antioxidant properties[9]. In one study on the potential of dragon fruit, it was concluded that it has hepatoprotective potential against acetaminophen induced liver injury[10]. The red dragon fruit peel was also tested and was found out to have a potential in reducing total cholesterol, trigliserida, and LDL-c and increasing HDL-c levels[11]. amazingly, the addition of white and red dragon fruit into yogurt enhanced the milk fermentation rate, lactic acid content, syneresis percentage, antioxidant activity, total phenolics content and organoleptic properties in yogurt[12].

Being rich in various nutrients, vitamins and minerals , and accordingly owing high medicinal values, dragon fruit is believed to be able to address a lot of fatal diseases[13]. Thus, this research will serve as confirmatory or new source of data that can be helpful in medicinal drug development and at the same time can serve as baseline data for other product development of the dragon fruit.

2. Objectives of the Study

Generally, this study aimed to determine the hepatoprotective effect of *Hylocereus polyrhizus* (dragon fruit) peel and flesh extracts to the hepatic liver damage of albino wistar rats induced with Carbon Tetrachloride (CCl_4). Specifically, the study aimed to:

1. Determine the phytochemical constituents of dragon fruit.

2. Compare the mean % changes for SGOT (serum glutamic oxaloacetic transaminase)and SGPT (serum glutamic pyruvic transaminase) levels obtained from pre and post medication with *Hylocereus polyrhizus* peel and flesh extract in albino wistar rats under the following:

a. negative control (induced CCl_4)

b. ethanolic extract;

c. crude extract;and,

d. positive control(sylimarin capsule)

2.1 Significance of the Study

The study is of significance to the following:

The Department of Science and Technology. The proposed study will give health and research institutions a scientific background and benchmark data on the hepatoprotective activity *of Hylocereus polyrhizus* (dragon fruit) as a basis in producing Anti-Liver disease drugs. It can also serve as basis in the discovery and development of alternative medicines for interested people in the drug business.

The Department of Agriculture. The conducted research study will enhance the demand for dragon fruit production and can serve as one of the bases of the Office in the identification of value added crops to be given priority in terms of production.

The Cagayan State University. The conducted research endeavor could be an additional input data to AACCUP and CHED accreditation requirements. It can also serve as evidence on the quality of researches undertaken by its students;

The community. The conducted research could result to the production of cheaper and affordable alternative medicines for the treatment of liver disease; and,

Future researchers. This research can serve as their reference in conducting parallel studies about the effect of *Hylocereus polyrhizus* (dragon fruit) on liver disease.

2.2 Scope and Delimitation of the Study

The study focused on the determination of the hepatoprotective effect of *Hylocereus polyrhizus* peel and flesh extracts to albino wistar rats with Carbon Tetrachloride (CCl_4) induced hepatic damages.

2.3 Time and Locale of the Study

This study was conducted at the Chemistry Laboratory of Carig Campus and the Cagayan Valley Herbal Processing Plant, Carig Sur Tuguegarao City, Cagayan from February to March 2018. The Dragon fruit was taken from Divisoria market, Tondo Manila, Philippines while the experimental animals were purchased from Gonzaga, Cagayan.

3. Materials

The materials, samples, apparatuses and chemical reagents that were used in the study were the following: *Hylocereus polyrhizus* peel and flesh,water, ethanol, carbon tetrachloride (CCl_4), Silymarin, Erlenmeyer flask, funnel, filter paper, pipette, aspirator, test tubes, beaker, mettle balance, spatula, aspirator, distilled water, mouse cages and rotary evaporator.

4. Experimental Design

The study made use of a complete randomized design (CRD). It included the extraction of plant materials followed by an evaluation of its hepatoprotective activity. The evaluation of the hepatoprotective activity of the *Hylocereus polyrhizus* peel and flesh extracts employed a pre-test – post-test experiment only. A total of 16 albino wistar rats were used in the study. The rats were subjected to acclimatization process under standard laboratory condition prior to the inducement of liver damage and administration of the *Hylocereus polyrhizus* extracts. A total of four groups were used and each group was replicated four (4) times. The rats were randomly numbered and were assigned to different treatments as follows:

Table 2: Lay out of the Study

Treatment 1	Received Carbon Tetrachloride (CCl_4) once in every 72 hours (0.5ml/kg body weight)
Treatment 2	Received Carbon Tetrachloride (CCl_4) once in every 72 hours (0.5ml/kg body weight), treated with ethanolic extract of *Hylocereus polyrhizus* once in every 24 hrs. (2500mg/kg body weight)
Treatment 3	Received Carbon Tetrachloride (CCl_4) once in every 72 hours (0.5ml/kg body weight), treated with Crude extract of *Hylocereus polyrhizus* once in every 24 hrs. (2500mg/kg body weight)
Treatment 4	Received Carbon Tetrachloride (CCl_4) once in every 72 hours (0.5ml/kg body weight), treated with sylimarin capsule once in every 24 hrs. (2500mg / kg body weight)

The four groups that were replicated four (4) times were assigned into cages with rice bran as substrate. The substrate was replaced every after two (2) days.

4.1 Acclimatization of the Experimental Animals

The sixteen (16) experimental albino wistar rats were acclimatized under standard laboratory condition. The Animal maintenance experimentation was carried out according to the rules and regulations of the Bureau of Animal and Industry. The ratio of the cages was 1:4.

The rats undergone fasting for 24 hours prior to the induction of CCl_4, and *Hylocereus polyrhizus* extracts.

4.2 Preparation of Extract

The collected fruits were cleaned and thoroughly washed with distilled water. The peel and flesh of the fruit were cut into tiny pieces before extraction.100g of the sample was mixed with the 200mL solvent 95% ethanol for 2-3 days. The extract were filtered and placed in a clean container. The flask containing the extracted compound was placed in a refrigerator to prolong the quality of the extract.

4.3 Phytochemical Screening of ethanolic extract of Dragon Fruit Peel

Phytochemical screening of the fruit material was done for:

1. **Detection of Alkaloids**
2. **Detection of Tannins.**
3. **Detection of Saponins**
4. **Detection of Flavonoids.**.
5. **Detection of Glycosides.**
6. **Detection of triterpenes.**

4.4 Inducement of Liver Damage

The albino wistar rats were induced to liver damage using Carbon Tetrachloride (CCl_4), in a dose of 0.5 ml/kg body weight (CHEMTREC USA, 2009). Using 1ml sterile syringe and gavage needle, the Carbon Tetrachloride (CCl_4) was administered orally and introduced directly on the gastrointestinal tract of the test animals to avoid spilling.

4.5 Blood Testing of the Experimental Rats

The experimental albino wistar rats that were induced to liver damage using Carbon Tetrachloride (CCl_4) were subjected to blood testing to determine the biochemical components particularly specifically serum glutamic axaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). The blood samples were extracted from the tail of the experimental rats.

4.6 Administration of the Silymarin

The commercial medicine was administered to the experimental animals in standard control group. The silymarin was administered orally and introduced directly on the gastrointestinal tract of the experimental animals to avoid spilling.

4.7 Administration of the Hylocereus polyrhizus Extract

The extracts from the dragon fruit peel and flesh were administered orally to the experimental animals at 2500mg/kg body weight for seven (7) days (Sook Yee Hor. Et.al, 2012). The extracts were dissolved with distilled water with a ratio of 105 grams extract in 520 ml water using Erlenmeyer flask and stirring rod. Using a three (3) ml of sterile syringe and a gavage needle, the extracts were administered directly to the gastrointestinal tract of the experimental animals to avoid spilling.

4.8 Blood Collection after the Administration of Hylocereus Polyrhizus Extract

After the inducement of CCl_4, blood sample of the experimental rats were collected from the tail for the liver function pre-test.

After the experiment, the albino wistar rats were deprived from food after seven days of treatment. Twenty-four (24) hours after the administration of the *Hylocereus polyrhizus* extracts, all the experimental rats were prepared for the liver function post test.

Collection of blood was also done from the tail. The collected blood samples were placed in sterile glass top tube and vertically placed to the poly tray.

4.9 Liver Function Test

For the liver function test, electrochemiluminiscence immuno assay was used. Biochemical components of blood, specifically serum glutamic axaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were measured. The blood samples that were centrifuged at 3400 rpm for 10 minutes to separate serum which were used for the assay of the biochemical indicators of liver damage. The analysis of blood biochemical composition was done at Best Diognostic Corporation ISO (9001:2008) at Rodamel Building, Carig Sur, Tuguegarao City, Cagayan.

5. Statistical Analysis

The experiment was analyzed using percentile estimate of the dependent variables and one way Analysis of

Variance (ANOVA) with 4treatments and 4 replicates each.

6. Results and discussion

This chapter discusses the results of the study, analysis, and interpretation of data. The summary of phytochemical constituents of ethanolic extract of Dragon Fruit (*Hylocereus polyrhizus)* is shown in Table 3. Based on the data gathered, ethanolic extract shows trace amounts in Alkaloids, Saponins, Glycosides, and Tannins. The extract also shows moderate amount of Triterpenes and Flavonoids.

Table 3: Summary of Phytochemical Constituents of Ethanolic Extract of Dragon Fruit (*Hylocereus polyrhizus)*

CONSTITUENTS DETECTED	RESULTS
Triterpenes	++
Flavonoids	++
Glycosides	+
Tannins	+
Alkaloids	+
Saponins	+

(+) Traces

(++) Moderate

The comparative mean percent (%) mean changesfor SGOT and SGPT levels from pre and post treatments with *Hylocereus polyrhizus* peel and flesh extract in Albino Wistar Rats is shown in Table 4.

The percent change for SGPT is -83.51% and for SGOT is -168.58% with rats induced with Carbon Tetrachloride which shows an increase values for both enzymes respectively; decreasedvalue of SGPT by 20.13% and SGOT by 55.27% is shown in T2; SGPT and SGOT also show decrease in value in T3 by 24.01% and 36.95% while the rats treated with sylimarin capsule (T4) show an increase SGPT by -32.55% and SGOT by -137.94%.

Table 5 displays the analysis of variance on the effectiveness of the different treatments to albino wistar rats. It shows the comparison on the blood samples of the rats subjected to the different treatments T1, T2, T3, and T4, respectively. The results show significant difference on the SGOT and SGPT levels of the rats with the different treatments.

This is because the p-value for the SGOT and SGPT tests are 0.00025 and 0.00325 respectively which are both lower than 0.01 level of significance. This further shows that there is significant difference in the effectiveness of the treatments to the damage of the rats' liver.

Table 4: Mean % changes SGPT and SGOT levels from pre and post medication of *Hylocereus polyrhizus* peel and flesh extract in Albino Wistar Rats

Treatment	**SGPT**				**SGOT**			
	Mean Before	**Mean After**	**Mean Difference**	**Mean Percent Changes**	**Mean Before**	**Mean After**	**Mean Difference**	**Mean Percent Changes**
T1 (0.5ml/kg body weight of CCl_4 without medication)	45.05	82.67	-37.62	-83.51	115.42	310.00	-194.58	-168.58
T2 (0.5ml/kg body weight of CCl_4 treated with 2500mg/kg body weight of Ethanolic extract of *Hylocereus polyrhizus)*	52.32	41.79	10.53	20.13	260.92	116.70	144.22	55.27
T3 (0.5ml/kg body weight of CCl_4 treated with2500mg/kg body weight of Crude extract of *Hylocereus polyrhizus)*	53.56	40.7	12.86	24.01	180.85	114.03	66.82	36.95
T4 (0.5ml/kg body weight of CCl_4 treated with 2500mg/kg body weight of sylimarin capsule)	42.15	55.87	-13.72	-32.55	114.27	271.89	-157.62	-137.94

Table 5: Analysis of Variance on the Effectiveness of the Different Treatments to Albino Wistar Rats

Variable	Sum of Squares Effect	Degrees of Freedom Effect	Mean Square effect	Sum of Square Error	Degrees of Freedom error	Mean Square error	Observed f	P value
SGOT	118476.2	4	29619.05	17103.09	12	1425.258	20.78153	0.000025
SGPT	3610.2	4	902.54	1486.87	12	123.906	7.28408	0.003235

Table 6 shows the pair wise comparison on the SGOT levels of the blood of the rats under the different treatments. T1 shows significantly higher SGOT as compared to the SGOT of the rats subjected to treatments T2 and T3 but with significantly the same level of SGOT with that of T4. In addition, T2 has comparable effectiveness as that of T3 since their difference is not significant. However, the SGOT of the rats under T2 is significantly lower than the rats under T4 since the p-value 0.000165 is less than 0.01 level of significance.

Table 6: Pair Wise Comparison on the SGOT Levels of the Blood of the Albino Wistar Rats under the Different Treatments

Treatments	1 M=310.00	2 M=116.70	3 M=114.03	4 M=271.89
T1		0.000022	0.000036	0.239946
T2	0.000022		0.927593	0.000165
T3	0.000036	0.927593		0.000253
T4	0.239946	0.000165	0.000253	

Table 7 displays the pair wise comparison on the SGPT levels of the blood of the rats subjected to the different treatments. It reveals that the SGPT of the rats under T1 is significantly higher than the SGPT of the rats under T2, T3, and T4 since the p-value for this comparison is less than 0.01 level of significance.

However, the SGPT levels of the rats under the treatments T2, T3 and T4 shows no significance. This means that the effectiveness of these T2, T3, and T4 are not significantly different.

Table 7: Pair Wise Comparison on the SGPT levels of the blood of the rats under the different Treatments

Treatments	**1** **M=82.670**	**2** **M=41.792**	**3** **M=40.700**	**4** **M=55.873**
T1		0.000428	0.000592	0.012181
T2	0.000428		0.899879	0.123568
T3	0.000592	0.899879		0.120881
T4	0.012181	0.123568	0.120881	

Figure 3 shows the comparison of the two different extracts with the commercial drug which is sylimarin capsule. The figure reveals that the crude extract is more effective than the ethanolic extract while the sylimarin capsule shows no preventive effect to the liver of the albino wistar rats induced with Carbon Tetrachloride (CCl_4).

Figure 3 Hepatoprotective Potential of the *Hylocereus polyrhizus* Crude and Ethanolic Extracts on CCl_4 InducedHepatic Damages in Albino Wistar Rats compared to Sylimarin Capsule

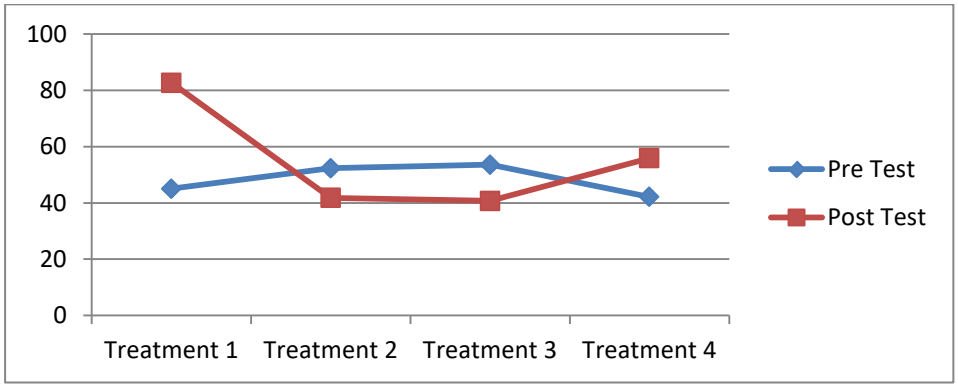

Figure 3: A. Pre and Post Test values of SGPT

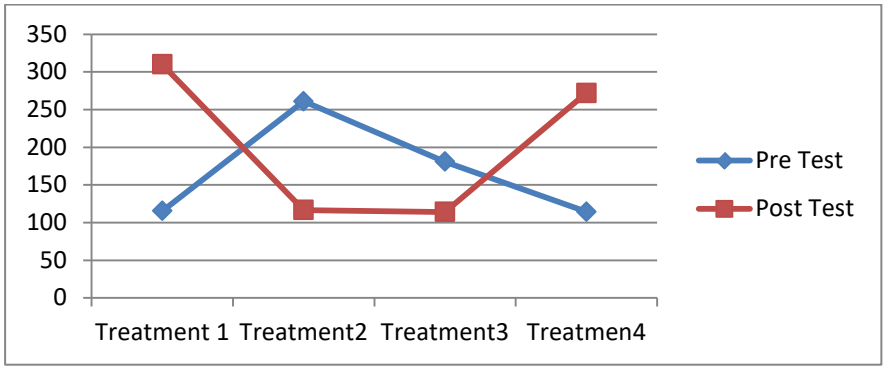

Figure 3: B. Pre and Post Test values of SGOT

Legend:

T1- Induced with CCl_4

T2- Induced with CCl_4, treated with ethanolic extract

T3 - Induced with CCl_4, treated with crude extract

T4- Induced with CCl_4, treated with sylimarin capsule

Carbon Tetrachloride (CCl_4) is one of the most powerful solvents toxic to the liver and is widely used in scientific research to assess liver damage and hepatoprotective agents. Indeed, carbon tetrachloride has been known for many years to be toxic to the liver.

The graph reveals that induction of Carbon Tetrachloride (CCL_4) in the gastrointestinal tract of the test animals caused them to suffer from toxic hepatitis. This was the result after 24 hours upon induction of Carbon Tetrachloride. Blood samples were collected to serve as the pre SGOT and SGPT values.

Toxic hepatitis is an inflammation of the liver in reaction to certain substances to which you are exposed. This kind of liver damage can be caused by alcohol, chemicals, drugs or nutritional supplements. This liver damage, in some cases, is developed within hours or days of exposure to a toxin but some cases also take months before it could be observed.

The hepatoprotective effect of the extract was done by administering it to the experimental animals after seven (7) days of CCL_4 inducement to see their effectiveness by getting their post SGOT and SGPT values.

After seven days of treatment, the results obtained showed positive response revealing that dragon fruit (*Hylocereus polyrhizus)* crude extract had a protective effect to save the liver from the continuing effect of the induced Carbon Tetrachloride (CCL_4) than that of the *Hylocereus polyrhizus* ethanolic extract. The dosage used for the test compounds were 2500mg/kg body weight but then, still, *Hylocereus polyrhizus* crude extract performed better as the hepatoprotective agent than that of *Hylocereus polyrhizus* ethanolic extract. However, the used commercial drug, sylimarin capsule, did not show any preventive effect to save the liver of the test animals.

7. Summary of Findings

The study was performed in the month of February until March 2018 with the general objective of determining the hepatoprotective effect of *Hylocereus polyrhizus* (dragon fruit) peel and flesh extract induced with Carbon Tetrachloride (CCl_4) to albino wistar rats.

The study was conducted by means of a completely randomized design with 4 treatments and 4 replications. The dragon fruit fruits, *Hylocereus polyrhizus,* were gathered from Divisoria Market, Tondo Manila, Philippines and were extracted using the facilities and equipments of Roco Laboratory, Cagayan State

University, Carig Campus, Tuguegarao City.

The bioassay was done at the Department of Health- Cagayan Valley Herbal Processing Plant (DOH-CVHPP), Carig Sur, Tuguegarao City in the month of March 2018.

The albino wistar rats were obtained from Gonzaga, Cagayan and then undergone acclimatization process before performing the test procedures. The rats were randomly numbered and assigned to different treatments and weighed. The rats were induced with carbon tetrachloride (0.5ml/kg body weight) in all treatments. After 24 hours, the pre blood samples were collected from the tail. Treatment 1 was continually induced using the dose 0.5ml/kg body weight once in every 72 hours for seven days. Treatments 2 and 3 were treated using the dragon fruit extracts with the dose 2500mg/kg body weight once in every 24 hours for seven days and treatment 4 was treated using the commercial drug, silymarin capsule using also the dose 2500mg/kg body weight once in every 24 hours for seven days. The rats were weighed daily during the experiment. After seven days of treatment, the blood was collected from the tail. The analysis of blood biochemical composition was done at Best Diagnostic Corporation (ISO 9001:2008) at Rodamel Building, Carig Sur, Tuguegarao City, Cagayan Valley.

Results revealed that the crude extract of *Hylocereus polyrhizus* peel and flesh extracts act as the most effective hepatoprotective agent among the groups.

8. Conclusion

Oral administration of *H. polyrhizus* extracts (2500mg/kg B.W) show a protective effect in rats poisoned with CCl_4 intake after 7 days because of its high antioxidant components coming from the above mentioned phytochemical components specially triterpenes and flavonoids which protect the liver from peroxidation of fats but sylimarin capsule has no protective effect against liver damage with corresponding rise of SGPT and SGOT markers.

9. Recommendations

Results obtained from the study had proved that *H. polyrhizus* extracts are effective in protecting the liver of the test animals against continuing damage when induced with CCl_4. For later studies, the following are recommended:

1. Compare the ethanolic and aqueous extracts using different dosages for possible better protective results.
2. Compare the extracts with another commercial drug which could have a greater impact to liver cells other than the used commercial drug in this study.
3. Development of products made up of the peel and flesh of the dragon fruit.

References

[1]. https://www.medicalnewstoday.com/articles/305075.php

[2]. https://www.webmd.com/digestive-disorders

[3]. http://www.haz-map.com/heptox1.htm

[4]. R. Suman Kumar,et.al(August 2013). Phytochemical Screening of Some Compounds from Plant Leaf Extracts of Holoptelea Integrifolia (Planch.) and Celestrus Emarginata (Grah.) used by Gondu Tribes at Adilabad District, Andhrapradesh, India.

[5]. Mishra, G., et. al. Hepatoprotective Potential of Ethanolic Extract of Pandanus Odoratissimus Root Against Paracetamol- Induced Hepatoxicity in Rats

[6]. Janey Alam, et. al. Hepatoprotective Potential of Ethanolic Extract of Aquilaria Agallocha Leaves Against Paracetamol Induced Hepatoxicity in SD Rats

[7]. Adeewale Adetutu and Olubukola S. Olorunnisola (2013). Hepatoprotective Potential of Some Local Medicinal Plants Against 2-Acetylaminoflourene-Induced Damage in Rat

[8]. https://www.livestrong.com/article/81272-dragon-fruit-nutrition/

[9]. Wee Sim Choo and Wee Khing Yong (2011). Antioxidant Properties of Two Species of Hylocereus Fruits.

[10]. Mihir Y. Parmar, et. al. Antioxidant and Hepatoprotective Potential of Dragon Fruit Extract on Opposition to Acetaminophen-Induced Liver Smash Up in Rats.

[11]. Hernawati, et. al. The Role of Dragon Fruit Peel (Hylocereus Polyrhizus) to Improvement of Blood Lipid Levels of Hyperlipidaemia Male Rats.

[12]. Zainoldin, K.H. and Baba, A.S. (2009). The Effect of Hylocereus Polyrhizus and Hylocereus Undatus on Physicochemical, Proteolysis, and Antioxidant Activity in Yogurt.

[13]. http://www.researchjournal.co.in/upload/assignments/12 267-271.pdf