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Ethanolic fruit extract of Zanthoxylum rhetsa mediates anti-cancer properties against Diethylnitrosamine (DEN) induced hepatocellular carcinoma (HCC) in mice

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Author Details

JESMIN SULTANA[a], ANKUR JYOTI DEKA[a], EYASIN ALI[a], ABDUL MALIK[b], SUHAIL AKHTAR[c], BARNALI DEKA[d], DIPANKAR BUJARBARUAH[e] and SUBHASH MEDHI[a*]

Authors Affiliations

[a]Laboratory of Molecular Virology and Oncology, Dept. of Bioengineering and Technology, Gauhati University, Guwahati, 781018, Assam, India
[b]Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arab
[c]A.T. Still University of Health Sciences, Kirksville, MO, USA
[d]Department of Chemistry, Barkhetri College, Narayanpur, Mukalmua, 781126, Assam, India
[e]Department of Zoology, Dimoria College, Khetri, Guwahati, 783403, Assam, India

Corresponding Author*
Dr. Subhash Medhi
(subhashmedhi@gauhati.ac.in)

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Abstract: Hepatocellular Carcinoma is a fatal disease and the third biggest cause of mortality from cancer. Because chemotherapy treatment may have a variety of adverse effects, the use of alternative cancer therapies has been a prominent focus of study. When compared to other drug discovery sources, traditional medicine has given several unique therapeutic molecules for preventative and curative therapy. This study examined the hepatoprotective potential of an ethanol-based extract of Zanthoxylum rhetsa fruits against Swiss albino mice's Diethylnitrosamine (DEN)-induced liver cancer. The animals were given oral dosages of 100 and 250 mg/kg body weight of the ethanolic extract, whereas DEN was delivered intraperitoneally once a week for 21 days. At the end of the treatment period, the animals were sacrificed, and liver tissue as well as blood were obtained to determine marker enzymes and oxidative stress indicators. On estimate, the treated group showed an improvement in the marker and antioxidant activity. These findings showed that the increased enzyme levels revealed a protective mechanism for Z rhetsa fruit extract in DEN-induced HCC in mice. The biochemical parameters were corroborated by histological examination of the animals' livers. As a result, ethanol extracts of Z rhetsa fruits have substantial anticancer effects.

Keywords: Zanthoxylum rhetsa, HCC, DEN, SGPT, SGOT, ALP, GSH, SOD, GST, CAT, and GPx.

ABBREVIATIONS:

HCC	Hepatocellular carcinoma
DEN	Diethylnitrosamine
BW	Body weight
SGPT	Serum Glutamate Pyruvate Transaminase
SGOT	Serum glutamic-oxaloacetic transaminase
ALP	Alkaline phosphatase
GSH	Glutathione
SOD	Superoxide diamutase
GST	Glutathione S-transferase
GPx	Glutathione peroxidase-1

INTRODUCTION

The global incidence of cancer has been steadily rising, which is a significant cause for alarm. The high death rate and absence of effective therapy have prompted researchers to conduct thorough investigations on chemoprevention [1]. The malignancies most often linked to diet include esophagus, stomach, colon, liver, and prostate cancer [2]. HCC is a liver-originating malignancy with the fifth-highest incidence rate globally. It ranks as the third most common cause of mortality, Amongst the other cancers such as lung, colorectal, and stomach cancer, HCC ranks in the third position globally in terms of its mortality rate[3]. HCC constitutes approximately 75% to 85% of the total incidence of primary liver cancer. This illness is a significant global problem since it causes millions of deaths worldwide each year[4, 5].

As per the findings of a survey carried out by GLOBOCAN, HCC ranked third among all causes of mortality worldwide in 2018, accounting for 781,631 fatalities or around 8.2% of the worldwide fatalities [6]. Approximately 80% of liver cancer cases primarily consist of HCC. In nations such as India, the reporting of cancer cases is challenging due to the predominant focus of cancer registries in urban areas. As a result, data regarding occurrences of hepatocellular carcinoma is substantially limited. Unpublished information from various Indian tertiary healthcare care institutions has revealed information about the escalating incidence of HCC each year [7]. Based on the data, the occurrence rates of HCC vary from 1 to 7.5 cases per 100,000 people. Among males, the rates range from 0.7 to 7.5, while among women, the rates range from 0.2 to 2.2. These prevalence rates are determined based on the age groups of individuals [8]. From the year 2012 to 2014, the highest incidence of liver cancer was observed in the Northeastern region of the country, specifically in Sikkim and Arunachal Pradesh, according to the Population-Based Cancer Registry, compared to other regions [9]. In 2010, there were around 14,000 recorded fatalities from liver cancer in India, specifically within a certain age group. The mortality rate for this kind of cancer was 6.8 (5.4-8.1) per 100,000 cases [10].

For generations, traditional medicine has used medicinal plants to treat a variety of illnesses, such as diabetes and cancer. The report contributed forth by the World Health Organization asserts that traditional knowledge-based medicinal plants constitute the primary healthcare system for over 80% of the global population. The plant Zanthoxylym rhetsa DC. is in the Rutaceae family and is in the genus Zanthoxylum. The plant is found in subtropical regions worldwide and has widespread distribution in India, Bangladesh, Sri Lanka, Vietnam, Indonesia, Malaysia, and China [11-13]. The plant is seen as a useful medicinal herb and is highly favored by various indigenous groups inhabiting the Indian subcontinent. It is extensively used as a conventional remedy and is associated with several ethnobotanical customs. Each component of the plant has distinct functions and has been highly esteemed for an extended period. The plant's different components have been utilized in Ayurvedic medicine and ethnomedicines for their beneficial activity against diabetes, muscle spasms, inflammation, pain, diarrhea, and water retention. Also, they are used to treat many different maladies [13-16].

Z. rhetsa seeds possess a strong and sharp flavor. The inhabitant tribes living in Nagaland and Arunachal Pradesh use the powdered seeds for piscicidal purposes. The Naga people in North-East India use the leaf extract as a deworming [17, 18]. Diverse communities employ the plant not solely for its culinary but also for its medicinal properties. The many components used include shoots, delicate foliage, and fruits. The pericarp of immature fruits has the fragrance and flavor of sweet orange peel and is used in the preparation of pickles. The fruits are often dehydrated and used as a seasoning and condiment, especially in fish and meat curries, to augment the taste and flavor. This practice is prevalent among the inhabitants of northeastern, western, and southern regions within India [19, 20]. As reported by Payum et, al 2013, the people of the Adi tribe utilize young shoots of Z. rhetsa in their diet[21].

This article aims to present the findings on the anticancer properties of the ethanolic extract derived from the fruits of Z. rhetsa. The study focused on its effects on hepatocellular carcinoma produced by NN-Diethylnitrosoamine (DEN) in Swiss albino mice.

MATERIALS AND METHODS

Chemicals

Diethyl nitrosamine was purchased from Sigma Aldrich Ltd to induce HCC. All other chemicals were purchased from Merck otherwise.

Collection, Identification, and extraction of plant materials

The Botanical Survey of India, Shillong, Meghalaya, India specialist verified the taxonomy of the Z.rhetsa fruits, which were obtained from the Sonapur tea estate in Guwahati, India with specimen number (BSI/ERC/Tech/2020/1315). The fruits were thereafter thoroughly cleaned, dried, and ground into coarse powder followed by a maceration procedure with ethanol for 48 h to extract its contents. The extract was then filtered and thoroughly desiccated under vacuum to remove any traces of moisture. The desiccated extract was preserved at a temperature of -20°C until it was needed for further procedures. The animal experimental studies used doses of 100mg/kg and 250mg/kg of the extract based on body weight.

Animal studies

For the animal experimental study, 4 to 6-week-old male and female Swiss albino mice with body weights ranging from 21 g to 26 g were used. The experimental animals were confined in rice husk-filled polypropylene enclosures. Cages were maintained and ventilated adequately at an ambient temperature of 25±2ºC. Throughout the experiment, the animals were subjected to a light cycle consisting of fourteen consecutive hours of light followed by 10 hours of darkness. The animals were fed a regular pelleted diet and had unrestricted access to water. The experimental investigation was carried out after obtaining consent from the animal ethics committee of the institution, under the reference number

IAEC/Per/2023-23/09-3. 20 mg/kg BW of the medication was given intraperitoneally. It was mixed with a 0.9% w/v solution consisting of sodium chloride.

Experimental Design

The entire animal experimental study was categorized into 5 groups (n=6) as shown in Table 1. Every group is comprised of the following:

Group 1: Positive Control with normal pellet diet.
Group 2: DEN Control with a weekly intraperitoneal (i.p.) administration of a single dosage of DEN to develop hepatocellular carcinoma (HCC).
Group 3: The reference medicine (Sorafenib) is administered orally once a day at a dosage of 30 mg/kg BW, along with a weekly single dosage of DEN.
Groups 4 and 5 were administered an oral dosage of Z. rhetsa extract (100 mg/kg & 250 mg/kg BW) once daily, along with one intraperitoneal injection of DEN each week.

The treatment period continued for 21 days, after which the experimental mice were sacrificed by cervical dislocation. To evaluate the biochemical indicators more thoroughly, the obtained blood samples were processed. The liver tissues were meticulously extracted, rinsed with normal saline to eliminate blood stains, and preserved in formaldehyde solution for further research purposes.

Table 1: Grouping of animals and treatment protocol

Group	Treatment
Group 1	Pellet + *ad.libitum*
Group 2	Negative control i.e. DEN treated (20 mg/kg BW) weekly for 21 days
Group 3	DEN (20 mg/kg BW weekly) + Standard drug (Sorafenib) of 30 mg/kg BW daily for 21 days
Group 4	DEN (20 mg/kg BW weekly) + 250 mg/kg BW extract daily for 21 days
Group 5	DEN (20 mg/kg weekly) + 100 mg/kg BW extract daily for 21 days

Biochemical parameters

The biochemical data, which were measured using conventional techniques, include marker enzymes and oxidative stress measures. Following Reitman and Frankel's instructions, serum liver enzyme markers SGPT and SGOT were measured [22]. Alkaline phosphatase (ALP) was determined using the methods of Linhardt K. and Walter K[23]., Blood glutathione (GSH) by Beutler[24], Glutathione peroxidase (GPx) by Hafeman et al.[25], Glutathione-s-transferase by Habig et al.[26], Superoxide dismutase (SOD) by Marklund and Marklund [27], and Catalase (CAT) according to Aebi[28].

Histopathological study

For histopathological studies, the extracted hepatic tissues from the experimental studies were fixed in formaldehyde solution and were further, dehydrated by ethanol solutions with concentrations ranging from 70%, 80%, 90%, up to 100%. Each dehydration step lasted for an hour. The tissues were then dehydrated using xylene and encased in paraffin wax. The implanted blocks were cut into sections with a thickness of 4 to 5 μm with the help of a rotary microtome. The tissues were immersed in a solution of 50% alcohol to initiate stretching and thereafter placed in a tissue flotation bath maintained at a temperature of 56° to 58° C. The slices were affixed to a sterile microslide and thoroughly dried. Before staining, the tissue sections were dewaxed by immersing them in xylene two to three times, each for 2 minutes. Then, water with decreasing concentrations of alcohol (100%, 90%, 80%, and 70%) was added, followed by water. Following hematoxylin and eosin staining, the specimen was examined via a light microscope, and photomicrographs were captured for documentation.

Statistical Analysis

To ascertain if there were statistically significant changes between the treatments in terms of different measures, the study employed a one-way ANOVA. A statistically significant difference is shown by a p-value of less than 0.05. To find significant discrepancies, certain treatment pairings are compared using the Tukey-HSD test and boxplots [29, 30].

RESULTS

3.1 Beneficial effect of the fruit extract of Z. rhetsa in reducing liver enzyme (SGPT and SGOT) levels and oxidative stress parameters in animals exposed to DEN

The impact of Z rhetsa fruit ethanolic extract on oxidative stress parameters and liver enzyme indicators in mice liver cancer induced by Diethylnitrosamine (DEN) is shown in **Table 2**.

Table 2:

Parameters	Normal	Treatment with DEN	Treatment with standard + DEN	DEN+ 250 mg extract	DEN+ 100 mg extract
SGPT	45.2 ± 5.3	96.7 ± 4.9****	59.6 ± 7.5	73.2 ± 4.4**	80.2 ± 9.2***
SGOT	37.7± 4.6	100.0 ± 10.1****	61.2 ± 5.3**	80.1 ± 4.4****	88.4 ± 6.2****
ALP	75.1 ± 5.6	114.9 ± 6.2***	88.2 ± 6.9	93.4 ± 9*	97.5 ± 3.7*
GSH	11.8 ±3.6	25.3 ± 5.3*	13.5 ± 4.6	18.1 ± 3.6	22.1 ± 4.3
SOD	59.8 ± 9.3	85.9 ± 8.9*	50.5 ± 7.7	55.4 ± 6.5	78.9 ± 9.4
GST	23.9 ± 5.3	72.6 ± 10.4***	75.5 ± 9.6***	60.6 ± 8.8**	83.9 ± 7.9****
CAT	206.87 ±14.4	285.00 ± 10****	255.33 ± 12.26**	263.60 ± 6.09***	287.32 ± 3.58****
GPx	21.3 ± 3.2	55.8 ± 6.4***	37.9 ± 5.6*	56.3 ± 6.2***	52.6 ± 7.7***

SD=Standard deviation, DEN=Diethyl nitrosamine, DEN treated=Swiss albino mice were treated with DEN (20 mg/kg), DEN + standard= mice treated with DEN and 30 mg/kg sorafenib, DEN + 250 mg extract= mice treated with DEN and crude ethanol extract of Z rhetsa fruits 250 mg/kg and DEN + 100 mg extract= mice treated with DEN and crude ethanol extract of Z rhetsa fruits 100 mg/kg. Mice given only water were categorized as a normal group. All the values are expressed in terms of mean±SD with n=6 in each group. ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05 are considered to be statistically significant and compared with control groups.

Beneficial effect of fruit extract of Z. rhetsa in reducing SGPT and SGOT levels in mice exposed to DEN

Fig. 1 depicted a significant rise in serum SGPT level in experimental animals exposed to DEN (P<0.0001), as compared to a control group of animals. However, when compared with DEN + Standard and DEN + 250 mg extract (P<0.01), the diseased group showed high significant rise in SGPT level, but no such remarkable decrease was observed in DEN + 100 mg extract. Also, a remarkable rise in SGOT levels was detected in DEN-exposed mice, as compared to a control group. When compared with DEN + Standard (P<0.001), DEN + 250 mg extract (P<0.05) but no significance was observed DEN and DEN + 100 mg extract **(Figure 2).**

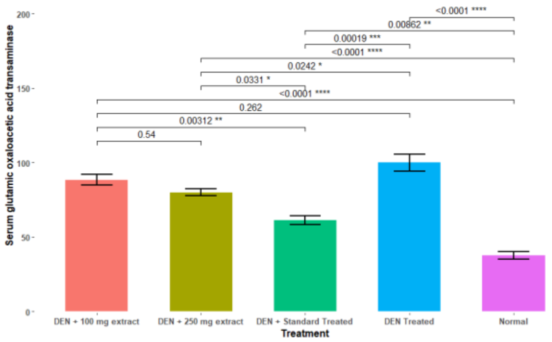

Figure 1: Pairwise comparison (Tukey-HSD) for Serum Glutamic Pyruvic Transaminase; P values: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 were considered to be statistically significant.

Beneficial effect of fruit extract of Z. rhetsa in reducing ALP levels in mice exposed to DEN

Figure 3 demonstrated a significant rise in the ALP enzyme level when the animals were exposed to DEN, compared to a control group of animals where the ALP levels were within normal range. Treatment with an extract dosage of 100 mg/kg BW however could not remarkably decrease the

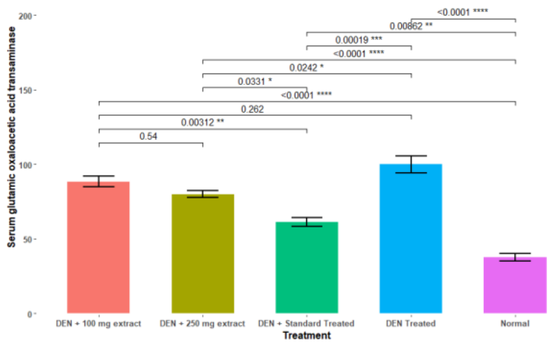

Figure 2: Pairwise comparison (Tukey-HSD) for Serum Glutamic Oxaloacetic Transaminase; P values: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 were considered to be statistically significant.

ALP level as compared to the control group. Subsequently, the animal groups treated with 250 mg/kg

BW of the extract and the Standard drug could significantly reduce the rise in the ALP level with p-value for DEN + Standard (P<0.001) and DEN + 250 mg (P<0.05) but significant reduction was less compared to the control group.

Beneficial effect of fruit extract of Z.rhetsa on GSH levels in mice exposed to DEN

From **Figure 4** it is seen that the GSH level significantly rose in the experimental animals exposed to DEN with a p<0.05. The animal groups when administered with both the dosages of the fruit extract of Z. rhetsa DEN + extract (100 and 200 mg/kg) BW could not remarkably reduce the GSH levels compared to a control group of mice. However, the group DEN + Standard (P<0.05), showed a significant reduction of GSH levels. Overall, it was observed that both the extract groups could not demonstrate a significant reduction in GSH levels.

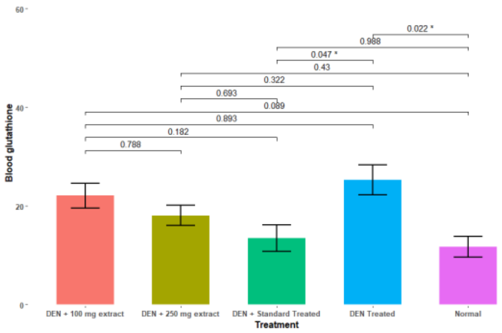

Figure 3: Pairwise comparison (Tukey-HSD) for Alkaline Phosphatase; P values:* P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 were considered to be statistically significant.

Beneficial effect of fruit extract of Z.rhetsa on SOD levels in mice exposed to DEN

From **Figure 5** it is observed that DEN exposed diseased animal group contributed to the rise in the SOD enzyme level compared to the control group without any treatment. Further, when treated with the extract doses of 100 mg/kg, the reduction in the SOD levels was not remarkable. When the extract dose was increased to 250 mg/kg BW, the SOD levels were reduced remarkably but the reduction was not that much compared to the control group. However, the animal group exposed to the standard drug reduced the SOD levels significantly which was comparable to the control group where no treatment was administered. Overall, it was observed the dosage of 250 mg/kg BW of the extract was more effective than the 100 mg/kg BW dosage, but the reduction was less significant than the group administered with the standard drug.

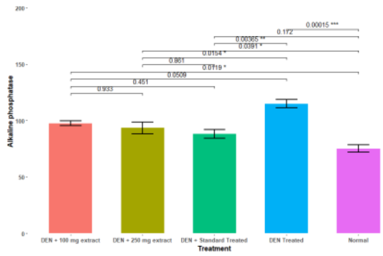

Figure 4: Pairwise comparison (Tukey-HSD) for Blood Glutathione; P values:* P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 were considered to be statistically significant.

Beneficial effect of fruit extract of Z.rhetsa on GST levels in mice exposed to DEN

From Figure 6 it is observed that DEN exposed diseased animal group contributed to the rise in the GST levels compared to the control group without any treatment. Further, when treated with extract doses of 100 mg/kg and 250 mg/kg BW, the reduction in the GST levels was not remarkable. Moreover, the animal group exposed to the standard drug could not significantly reduce the GST levels compared to the control group where no treatment was administered. Overall, it was observed that none of the extract dosages 100mg/kg, 250 mg/kg BW as well as the standard drug was not very effective in reducing GST levels.

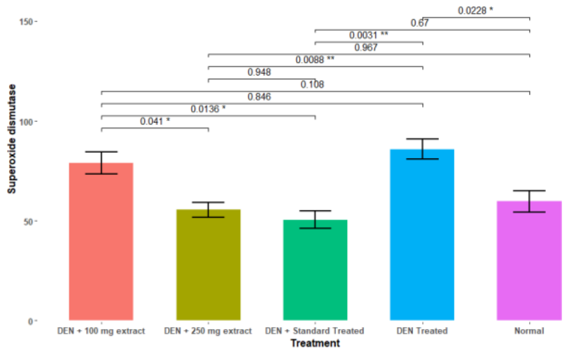

Figure 5: Pairwise comparison (Tukey-HSD) for Superoxide Dismutase; P values* P<0.05, ** P<0.01, *** P<0.001, **** P<0.00 were considered to be statistically significant.

Beneficial effect of fruit extract of Z.rhetsa on Catalase levels in mice exposed to DEN

From Figure 7 it is observed that DEN exposed diseased animal group contributed to the rise in the Catalase levels compared to the control group without any treatment. Further, when treated with extract doses of 100 mg/kg and 250 mg/kg BW, the reduction in the Catalase levels was not remarkable. Moreover, the animal group exposed to the standard drug could somewhat significantly reduce the catalase levels, but the reduction was less significant compared to the control group where no treatment was administered. Overall, it was observed that none of the extract dosages 100mg/kg, and 250 mg/kg BW could not effectively reduce the Catalase levels.

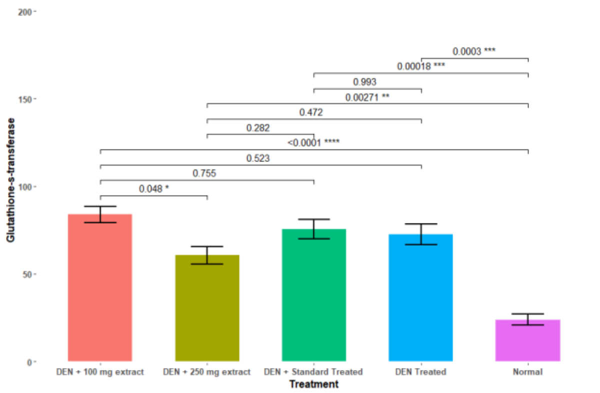

Figure 6: Pairwise comparison (Tukey-HSD) for Glutathione-S-Transferase; * P<0.05, ** P<0.01, *** P<0.001, **** P<0.000

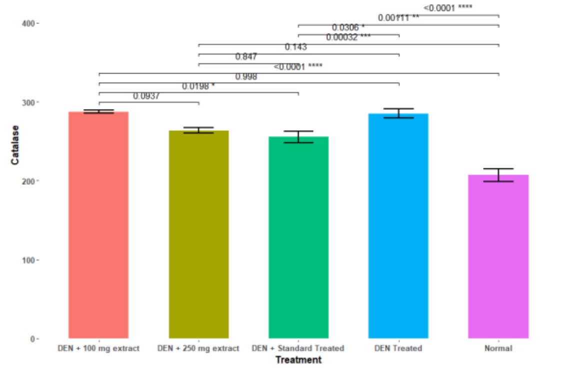

Figure 7: Pairwise comparison (Tukey-HSD) for Catalase; P values: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 were considered to be statistically significant.

Beneficial effect of fruit extract of Z.rhetsa on GPx levels in mice exposed to DEN

From Figure 6 it is observed that DEN exposed diseased animal group contributed to the rise in the GPx levels compared to the control group without any treatment. Further, when treated with extract doses of 100 mg/kg and 250 mg/kg BW, the reduction in the GPx levels was not remarkable. However, the animal group exposed to the standard drug could significantly reduce the GPx levels compared to the DEN-exposed group where GPx levels were high. Overall, it was observed that the extract dosages of 100mg/kg and 250 mg/kg BW were not very effective in reducing GPx levels.

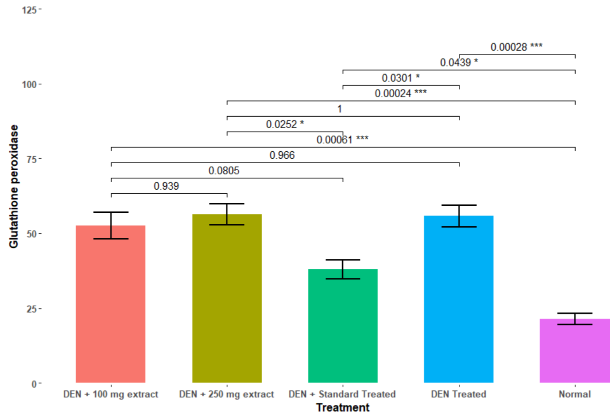

Figure 8: Pairwise comparison (Tukey-HSD) for Glutathione peroxidase; P values: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 were considered to be statistically significant.

Beneficial effect of fruit extract of Z. rhetsa on liver histology of mice exposed to DEN

On histopathological examination, it was observed that in the control group, the hepatocytes seemed normal, but the DEN-treated group exhibited significant changes, as demonstrated by congested vasculature in the hepatic parenchyma and diffuse regions of hepatocyte edema. Focal degenerative alterations signify limited regions of degeneration or injury in the liver tissue which was due to exposure to DEN. These findings suggest that pathological changes in the liver tissue, due to DEN exposure, are causing the development of HCC. On administration with the extract doses of 250 mg/kg BW and the standard drug Sorafenib, there was an improvement in the damaged hepatocytes. The images are shown in Figures 9A to 9E.

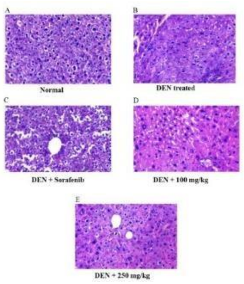

Figure 9: Beneficial effect of fruit extract of Z.rhetsa on liver histology, (A) Normal group, (B) DEN treated group, (C) DEN + Sorafenib treated group, (D) DEN + 100 mg/kg BW, (E) DEN +250 mg/kg BW.

DISCUSSION

This study highlights the possibility of scientific evidence of a traditional knowledge-based medicinal plant, Zanthoxylum rhetsa, as a remedy for exerting an anti-cancer effect.

Over 18,000 fatalities from liver cancer occur each year in the United States, affecting about 22,000 people [31]. In India, around 14,000 deaths were recorded in 2010, with a death rate of 6.8% (5.4–8.1) per 100,000 individuals [10]. Previously, no medication has been approved by the US FDA expressly to be used in the therapy of liver cancer. Nevertheless, in 2007, the United States Food and Drug Administration (FDA) approved Sorafenib, a targeted small-molecule drug, as the first-ever treatment for advanced liver cancer. Until now, Sorafenib has been the only preferred remedy for the first targeted therapy in the treatment of progressing HCC [32]. Hepatocellular carcinoma is the fifth most prevalent kind of cancer globally and the third greatest cause of cancer-related deaths. [31]. The 5-year survival rate for patients with HCC was predicted to be 18%. The explanation is attributed to the fact that only 30%–40% of the cases are detected in the initial stages, but the majority of cases are recognized after the illness has advanced in the liver, leading to vascular invasion and metastasis [33, 34]. Despite recent progress, such as the development of drug screening techniques and new technologies, HCC continues to pose a significant challenge in terms of effective treatment.

A complex disease, liver cancer is typified by many primary hepatic malignancies, including hepatoblastoma, hemangiosarcoma, and cholangiocarcinoma. However, HCC, which accounts for 70–85% of cases, remains the most common kind [35, 36].

Elevated bloodstream concentrations of cytoplasm and mitochondrial enzymes occur once hepatocytes are damaged or harmed. Quantifying the concentrations of these enzymes serves as a very responsive biomarker of liver injury [37]. An increase in enzyme levels may indicate liver dysfunction and is often used as a diagnostic indicator for liver illnesses. Although antioxidants are essential for safeguarding cells against oxidative harm, an excessive number of antioxidants may impede some protective activities of the body, such as the normal elimination of damaged or malignant cells by the immune system [38]. Nevertheless, it is believed that cancerous cells often exhibit elevated amounts of antioxidants, which serve to shield them from apoptosis triggered by oxidative stress [39, 40]. However, momentarily diminishing these defensive mechanisms may render the cancer cells more vulnerable to apoptosis-inducing treatments, such as radiation and chemotherapy [40]. This approach may be used to enhance the efficacy of cancer therapy.

DEN, a well-recognized environmental carcinogen, has a straightforward metabolic route and strong carcinogenic activity and is capable of causing liver cancer[41]. During the onset of laboratory-simulated hepatic carcinogenesis, DEN has been seen to cause a slight increase in the rate of proliferation of hepatocytes. The presence of early preneoplastic foci has confirmed this[42, 43]. DEN is used as a powerful hepatocarcinogen in laboratory animals to develop hepatocellular carcinoma (HCC)[44]. A study on the protective effects of an extract from Cassia fistula Linn. leaves against oxidative stress and DEN-induced HCC in rats given ethanol pretreatment was carried out by Kannampalli P in 2010[45]. Both ethanol- and DEN-treated groups of rats were orally supplied with the leaf extract for 30 days[45]. The results indicated that the treated group saw considerable improvements in liver marker enzymes and oxidative stress. As a consequence, the majority of the parameters evaluated showed improvement, similar to the effects of the conventional pharmacological treatment, silymarin[46]. The study showed that Ayurvedic milk extract from Semecarpus anacardium nuts could help fight cancer in Wistar rats that had been given HCC [46]. The findings demonstrated a favorable link between the Ayurvedic milk extract and the effects of doxorubicin, which was used as the standard treatment.

Within the realm of Indian medicine, some plants are renowned for their therapeutic properties and are said to alleviate liver ailments. Extensive research has been undertaken on cancer utilizing conventional medications to investigate and develop new therapeutic drugs, which are effective and do not exhibit severe side effects often associated with existing chemotherapeutic treatments[47].

Zanthoxylum rhetsa (Roxb.) DC, commonly referred to as Zanthoxylum budrunga and belonging to the Rutaceae family, is an aromatic tree of medium size characterized by the presence of cone-shaped thorns on the bark of both trunk and branches. This traditional, knowledge-based medicinal plant is renowned for its therapeutic applications. The desiccated fruits of the plant have historically been used as a traditional spice, while both the leaves and the fruit themselves are employed in many culinary concoctions, including both sweet and savory foods[17, 48].

The primary aim of our investigation is to evaluate the possible anti-carcinogenic benefits of the extract at various dosages. Our investigation included the administration of DEN, a carcinogen, to mice. The primary objective was to assess the effects of these therapies on the progression of HCC in mice.

The liver's biochemical marker enzymes provide insights into the liver's functionality. These indicators play a crucial role in the diagnosis and surveillance of many liver disorders and illnesses. The indicators included in our research are serum glutamic pyruvic transaminase, serum glutamic oxaloacetic acid transaminase, and alkaline phosphatase. The oxidative stress enzymes found in the liver are essential for maintaining the balance between the body's ability to neutralize reactive oxygen species (ROS) and their production. Oxidative stress may arise when there is an imbalance, leading to the development of several liver disorders and ailments. Numerous indicators of oxidative stress were found in the study, including glutathione peroxidase, catalase, superoxide dismutase, glutathione-S-transferase, and blood glutathione.

The average levels of SGPT, SGOT, ALP, blood glutathione, GST, catalase, and GPx were higher in the "DEN-exposed" group compared to the "normal" group. The group treated with "DEN + Standard Treated" exhibited a decrease in SGPT levels in comparison to the "DEN Treated" group. In addition, the administration of the herbal extracts "DEN + 100 mg extract" and "DEN + 250 mg extract" demonstrated a further reduction in the levels.

The group treated with DEN showed significantly increased average levels of SOD compared to the group labeled "normal." The administration of herbal extracts ("DEN + 100 mg extract" and "DEN + 250 mg extract") resulted in a reduction in superoxide dismutase (SOD) levels.

The low p-values (all p 0.001) obtained from ANOVA testing demonstrated significant variations across the treatment groups for all the biochemical indicators. These disparities indicate that the different therapies had a significant effect on these indicators in mice with DEN-induced HCC.

Collectively, the data indicates that the administration of treatment, including conventional medication and herbal extracts, had advantageous outcomes on various biochemical indicators. This resulted in a decrease in markers linked to liver impairment and oxidative stress, while simultaneously augmenting markers associated with antioxidant function.

The histological assessment was conducted using hematoxylin and eosin staining. In the control group, the hepatocytes seemed normal, but the DEN-treated group exhibited significant alterations, including congested vasculature in the hepatic parenchyma and diffuse regions of hepatocyte edema. A small number of hepatocytes had a prominent basophilic nucleus. Additionally, there were localized areas of hepatocyte enlargement and multifocal degenerative alterations in the liver tissue. The observation of mononuclear cell infiltration in the liver tissue was noted.

The presence of congested vasculature in the hepatic parenchyma indicates an abnormal buildup of blood inside the blood capillaries of the liver tissue. Hepatic parenchyma is the term used to describe the functioning tissue of the liver. Focal degenerative alterations signify limited regions of degeneration or injury in the liver tissue. Likewise, localized regions of cellular swelling may occur as a reaction to injury or stress. Basophilic nucleus refers to the staining pattern of the nucleus, in particular hepatocytes, which may suggest alterations in the cell's activity or function. Enlarged nuclei may also indicate cellular stress or alterations. Multifocal degenerative changes occur due to the presence of cellular debris or metabolic abnormalities. These findings suggest that pathological changes in the liver tissue, possibly brought on by the injection of DEN, are causing the development of HCC.

Our data indicate that there was a slight degree of change seen in the treated group when they received the conventional treatment or a 250 mg extract. The ethanol extract of Z. rhetsa had a hepatoprotective effect, as shown by a significant reduction in cellular edema, multifocal degenerative alterations, and infiltration of mononuclear cells.

CONCLUSION

According to the current investigation, administering an ethanol-based extract of Z rhetsa fruits to mice with generated HCC at dosages of 100 and 200 mg/kg BW for 21 days had a dose-dependent anti-cancer effect. However, further research is required to identify the specific active component in Z rhetsa and its mechanism of action to validate its effect.

Conflict of Interest: There is no conflict of interest in the research.

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Figure legends

Figure 1: Pairwise comparison (Tukey-HSD) for Serum Glutamic Pyruvic Transaminase;

P values: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 were considered to be statistically significant.

Figure 2: Pairwise comparison (Tukey-HSD) for Serum Glutamic Oxaloacetic Transaminase;
P values: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 were considered to be statistically significant.

Figure 3: Pairwise comparison (Tukey-HSD) for Alkaline Phosphatase; P values:* P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 were considered to be statistically significant.

Figure 4: Pairwise comparison (Tukey-HSD) for Blood Glutathione; P values:* P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 were considered to be statistically significant.

Figure 5: Pairwise comparison (Tukey-HSD) for Superoxide Dismutase; P values* P<0.05, ** P<0.01, *** P<0.001, **** P<0.00 were considered to be statistically significant.

Figure 6: Pairwise comparison (Tukey-HSD) for Glutathione-S-Transferase; * P<0.05, ** P<0.01, *** P<0.001, **** P<0.000

Figure 7: Pairwise comparison (Tukey-HSD) for Catalase; P values: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 were considered to be statistically significant.

Figure 8: Pairwise comparison (Tukey-HSD) for Glutathione peroxidase; P values:* P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 were considered to be statistically significant.

Figure 9: Beneficial effect of fruit extract of Z.rhetsa on liver histology, (A) Normal group, (B) DEN treated group, (C) DEN + Sorafenib treated group, (D) DEN + 100 mg/kg BW, (E) DEN +250 mg/kg BW.

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