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Assessment of the Oral Toxicity of Anogeissus pendula Plant Extract in Experimental Rats

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ABSTRACT

Background: We are the first to report on a safe dosage range via oral administration of whole plant *Anogeissus pendula*. According to the Organization for Economic Co-operation and Development (OECD) guidelines 423 and 407 respectively, acute and subacute oral toxicity studies were evaluated.

Material and Methods: Acute oral Toxicity was evaluated by oral administration of the extract as a single dosage (2000 and 5000 mg/kg b.w.) at 72 hours detrimental influences (including unwanted influence), death and behaviour have been observed up to 14 days. In the sub-acute oral studies, the extracts were administered orally at each of the three concentrations (500, 1000 and 2000 mg/kg b.w.) over 28 days, with changes observed during this recording period.

Result and Discussion: At a dose of 2000 mg/kg b.w. mild drowsiness and lethargy were reported in the treatment group extract; no behavioural change or symptoms at all. Administration of the liver extracts (2000 mg/kg b.w.) significantly enhanced the liver function indices.

Conclusion: The extract in the oral route did not show any sign of toxicity.

Keyword: *Anogeissus pendula, Ethanolic extract, Oral Toxicity, Sub-acute toxicity study, Acute toxicity study, Institutional Animal Ethics Committee (IAEC)...*

1. INTRODUCTION

Plants have long been esteemed for their therapeutic qualities, providing natural treatments for a wide array of health issues, since they are generally accepted, efficient, economical, safe, and low-cost. The public's usage of herbal products has increased due to the perception that these formulations are natural and harmless, making them useful in curing a wide range of illnesses[1]. According to reports from other studies, certain herbal therapies may contained heavy metals, aflatoxins, and dangerous germs[2]. Herbal medications are derived from nature and are generally free from the harmful or unpleasant adverse effects often associated with chemically synthesized compounds used in standard treatments. The toxicity of traditional herbal remedies should be investigated, as well-researched and produced mainstream orthodox medications, albeit this is not always the case [3]. Instead, it ought to be applied to authorized and legitimate herbal medicine products. Because of this, people who take herbal medicine typically concentrate on the product's medicinal qualities instead of their detrimental effects on different organs; nonetheless, toxicity studies should also be emphasized [4].

Anogeissus pendula, the plant species commonly referred to as "Dhok," is a member of the Combretaceae family. Even though it is occasionally called a "Dhok," it is not the same as the well-known flowering plant of the same name. Pendula is used orally to enhance gut flora, bowel difficulties, and heart-related ailments, and to give nourishing advantages for the heart due to its soothing and antibacterial characteristics. It may also aid in the healing of episiotomy wounds, leg ulcers (neuropathic and venous), gingivitis, radiation-induced mucositis, vaginal candidiasis, and radiation dermatitis. It has anti-inflammatory, anticancer, and cytotoxic qualities [5]. The possibility that toxicity would affect an entire organism—a plant, bacteria, or animal is the end outcome [6]. Numerous medical institutions worldwide are taking an interest in the topic of therapeutic plants [7]. Moreover, investigations on the pertinent dose toxicity of medicinal herbs are still few. This means that you cannot just take the fact that a plant has been used traditionally for biological purposes as proof that it would be safe [8]. So, we plan to study both the acute and subacute oral toxicity as per OECD guidelines performed on laboratory animals led us to conclude there is no accurately defined allowable dose range for an ethanolic extract from the *Anogeissus pendula*

plant. Given the information available at the time, this was a fair conclusion to reach

2. MATERIAL AND METHODS

Plants Material: Collection and Authentication

The *Anogeissus pendula* plant was gathered in July 2024 in Bhiwadi, Rajasthan. The plants were air dried and washed. The plant was authenticated at CSIR- National Institute of Sciences Communication and Policy Research in Charge in Delhi, India and issued with a voucher code (NIScPR/RHMD/Consult/2022/4145-46-1).

Extraction of Plant Material

The plant was carefully harvested from its natural habitat in Bhiwadi, Rajasthan. The plants were dried in the shaded area to secure the active metabolites. After drying the plant material was converted into a fine coarse powder, passed into the sieved no 18 for uniformity. Then the plant material was defatted with the help of Petroleum ether (40-60ºC) for 4 hours by using the Soxhlet apparatus. This defatted dry plant powder (Marc) was extracted by a Soxhlet device with solvents in the order of polarity, including ethyl acetate, ethanol and water respectively. The concentrated solutions were then evaporated in a rotatory evaporator until burnt, to remove the remaining solvent. Extracts were stored at 2-8ºC in the refrigerator.

Animals

For the oral toxicity evaluation, healthy Wistar rats weighing 200–225 g was sourced from the animal house at IFTM University, Moradabad. The rats were acclimatized for 7 days under standard laboratory conditions, maintained at a 12:12-hour light/dark cycle, a temperature 28°C with 70% relative humidity (RH). They were provided with a standard pellet diet and RO water *ad libitum* throughout the study. All experimental procedures were conducted with approval from the Institutional Animal Ethics Committee (IAEC) and under the supervision of the animal house in charge

Acute Oral Toxicity Study

Acute oral toxicity was assessed following OECD Guideline 423. The rats were divided into 4 groups (n=6) for each group animals were nulliparous. According to the OECD guidelines for acute oral toxicity, a single dose of 2000 mg/kg body weight was administered. There is an additional dosage of 5000 mg/kg included to clarify the toxicity, and it should be considered as a higher dosage. On the other hand, the positive control group received 1000 mg/kg b.w. of 10% w/w liquid paraffin treatment. On the first day of the study, all doses were given one time orally to the rats. The rats were observed for the initial 24 hours and monitored closely through the first six hours. Every rat was weighed and assessed for other parameters such as biochemical and haematological as per OCED guidelines after 14 days. This classification permits a comparison of the effects of different treatments and dosages on the outcomes measured.

Sub-acute oral toxicity study

A sub-acute oral toxicity study was conducted following OECD Guideline 407. The rats were divided into five groups, each consisting of ten animals (five males and five females). All animals were orally administered EEAP for 28 days while under continuous webcam monitoring. All procedures and observations were conducted in accordance to OECD guideline 407 [12]. The groups in this study were classified according to their sample treatment and respective dose regimens. Group (I) served as control with no treatment administered, whereas Group (II) was given 2000 mg/kg b.w. of liquid paraffin. On the other hand, Groups (III), (IV), and (V) were subjected to varying doses of EEAP, namely 500mg/kg, 1000mg/kg and 2000 mg/kg body weight, respectively. This classification allows a comparative analysis of how different treatments and dosage levels affect key study parameters, such as survival rate, body weight, and organ weight etc.

Effect of EEAP on physiological parameters

At the end of the sub-acute oral toxicity study, the rats were euthanized with the help of Ketamine HCl (80mg/kg i.p.) and the blood sample was collected through cardiac puncture. The blood sample collected was analyzed for haematological and biochemical parameters. The level of haematological and biochemical was found to be within the normal range except the SGOT and SGPT in 1000 mg/kg and 2000 mg/kg[13].

3. RESULT AND DISCUSSION

Acute Oral Toxicity Study

The result of the acute oral toxicity study was evaluated as per OECD guidelines, two-dosage regimen was employed for the investigation (2000mg/kg and 5000mg/kg). The findings suggest that there are no signs of toxicity, mortality and morbidity as well. The general behavior during the first 4 hours is normal which is up to 14 days. So, the LD_{50} would be greater than 5000mg/kg b.w., suggesting that the EEAP was safe at this dosage.

Observation of General Behavior

The results of the study, showed that the none of the groups during the observation of general behaviour such as body weight, temperature, food intake, urine, respiration rate, or adverse oral effects does not show any sign of changes. There were no fatal side effects reported, and there was no sedation or change in eye colour. Nonetheless, Group IV (5000 mg/kg

EEAP) showed minor sleepiness, which may have been caused by the greater dose. All things considered, the therapy was well-tolerated, and there were no significant differences between the groups in the other physiological parameters assessed. This suggests that further research is necessary to determine the significance of mild sleepiness and the pharmacological effects of EEAP at higher dosages.

Rate of Mortality (%)

In the research, the death rates were evaluated for each of the four categories. The group (I) that was not given any therapy had a 0% death rate. Similarly, group (II), which received a 2000 mg/kg dosage of Simple Paraffin, similarly showed no mortality. With a death rate of 0%, Group III, administered 2000 mg/kg b.w. of EEAP, exhibited no mortality. Notably, group (IV), which received 5000 mg/kg of EEAP, a greater dosage, also showed no mortality. All groups combined showed a 0% mortality rate, meaning that neither the control group nor the therapies caused any deaths.

Effect of EEAP on sub-acute oral toxicity study

OECD Guideline 407 sub-acute oral toxicity study was conducted. The extract was orally given in doses of 500, 1000, and 2000 mg/kg body weight from Day 0 until the end of Day 28. The rats showed no adverse signs or symptoms throughout the study period. They behaved normally and there were no fatalities. Collectively, these findings indicated that EEAP was safe for more pharmacological tests forward.

Effect of extract on the relative weights of vital organs

At the termination of the study, the animals were euthanized according to the guidelines of the OECD or CPCSEA. The vital organs were carefully excised and weighed. The findings indicate that there were no significant alterations in the relative organ weights compared to the control group. (data are shown in Table 1)

Table 1: Average organ mass (g) of rats

ORGAN	CONTROL	PARAFFIN	EEAP (500 mg/kg body weight)	EEAP (1000 mg/kg body weight)	EEAP (2000 mg/kg body weight)
HEART	3.32±0.06	3.43±0.01	3.35±0.18	2.63±0.35	3.45±0.18
LIVER	6.52±0.22	6.36±0.52	6.66±0.20	6.44±0.51	7.25±0.35
KIDNEY	3.22±0.11	3.24±0.29	3.42±0.27	3.36±0.12	3.41±0.29
SPLEEN	0.30±0.18	0.31±0.15	0.31±0.17	0.33±0.12	0.30±0.11
LUNGS	5.14±0.13	5.25±0.54	5.55±0.47	5.37±0.28	5.07±0.18

Data are presented as the mean ± SEM (n=6) from six rats

Effect of EEAP on Hematological and Biochemical Parameters

The safety profile of Ethanolic Extract of Anogeissus pendula (EEAP) was investigated in the subacute oral toxicity study according to OECD Guideline 407. Oral doses of the extracts were 500, 1000 and 2000 mg/kg body weight. Haematological and biochemical parameters did not show any substantial change, indicating safety. Nevertheless, there were observed mild increases in SGOT and SGPT levels at higher doses (1000 and 2000 mg/kg body weight) as presented in Table 2. These modifications were not sufficient to indicate very strong toxicity.

Table 2: Effect of Oral EEAP Administration on Hematological and Biochemical Parameters in Rats

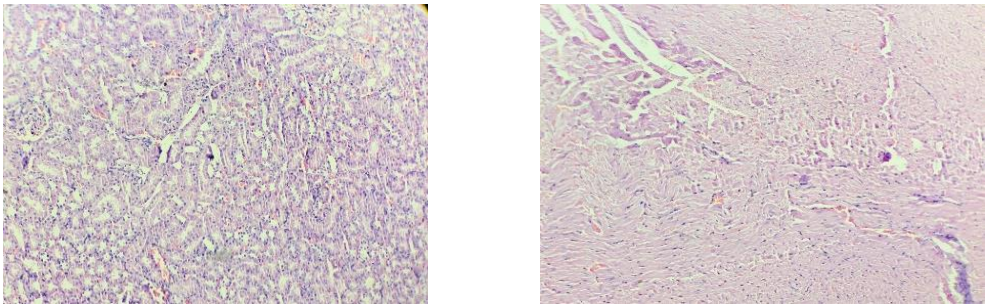
PARAMETERS	CONTROL	PARAFFIN TREATED	EEAP (500 mg/kg body weight)	EEAP (1000 mg/kg body weight)	EEAP (2000 mg/kg body weight)
HAEMOGLOBIN	12.43±0.20	12.13±0.29	12.40±0.44	12.34±0.27	12.05±0.14
LYMPHOCYTE	50.86±2.004	43.44±0.13*	50.67±0.79	50.13±2.12	50.13±0.12
MONOCYTE	7.17±0.31	7.04±0.25	7.81±0.19	7.28±0.33	7.28±0.15
NEUTROPHILS	26.41±0.22	29.40±0.69	27.75±0.22	24.32±0.42	25.10±0.70
RED BLOOD CELL	7.47±0.02	8.53±0.50	7.45±0.37	7.30±0.35	7.11±0.13.

WHITE BLOOD CELL	11.08±0.14	13.15±0.03	11.08±0.18	10.83±0.78	10.01±0.41
SERUM GLUTAMIC PYRUVIC TRANSAMINASE	57.60±0.88	60.25±0.18	60.13±0.613	65.08±0.74*	68.27±0.35*
TOTAL CHOLESTEROL	127.400±2.15	119.420±1.217	117.220±4.331	121.330±3.035	124.826±4.103
SERUM GLUTAMIC-OXALOACETIC TRANSAMINASE	32.11±1.37	39.13±0.10*	32.11±0.25	38.10±0.46*	39.11±0.21*
CREATININE	0.325±0.06	0.162±0.52*	0.411±0.07	0.470±0.09	0.655±0.02
TOTAL PROTEIN	6.22±0.04	7.03±0.15	7.31±0.05	7.05±0.73	7.12±0.82
BILIRUBIN	0.64±0.14	0.24±0.36	0.73±0.13	0.55±0.14	0.68±0.16

Mean ± SEM, followed by turkey test; *P>0.5

Histopathological Examination

Histological examinations on kidney specimens that were treated with EEAP and base normal also yielded the results presented in Figure 1, revealing that glameli and tubules exhibited intactness in a rat kidney. During the histopathological examination slight increase in tubular necrosis as well as in lymphocytic infiltration in rats who were treated with 2000mg/kg b.w. of EEAP. Similarly, in the liver section slight necrosis was seen in the hepatocytes in the 2000mg/kg dosage group. Other groups do not show any signs of toxicity which means the plant extract was used in any other pharmacological evaluation.



Kidney **Liver**

Figure 1: Photomicrographs of Histopathological slides of vital organs at 100X

4. CONCLUSION

The use of plant-based medications in healthcare is becoming more popular because they are assumed to be safer than conventional medicines and have fewer side effects. Bioactive agents derived from herbal flora are widely considered safe and necessary for the treatment of chronic diseases. The toxicity of these treatments was not investigated. The acute and subacute oral toxicity profile of *Anogeissus pendula* ethanolic extract (EEAP). Lethal Doses: 2000 mg/kg, 5000 mg/kg (OCED Guidelines; acute toxicity study) No toxicity was observed over 74 h of assessment, resulting in an LD50>5000 mg/kg signifying the safety of EEAP at this dosed level. The doses tested were 500, 1000 and 2000 mg/kg for the subacute study. The organ weights (liver, kidneys, spleen, heart and lungs) were comparable to controls suggesting that there is no organ toxicity. Hematological and biochemical parameters remained at normal levels (after 28 days). Although the markers of liver function (SGOT, SGPT) were slightly elevated, elevation was not sufficiently significant to indicate real or life-threatening hepatic toxicity. The increase may be caused by a phytochemical substance that can be ability poison at high levels. These results demonstrate that EEAP has no evidence of acute or subacute oral toxicity and may provide a rationale for further in vivo studies and clinical application.

5. CONFLICT OF INTEREST

None

ABBREVIATIONS

OECD: Organisation for Economic Co-operation and Development.

IAEC: Institutional Animal Ethics Committee

CSIR: Council of Scientific & Industrial Research

EEAP: Ethanolic Extract of *Anogeissus pendula*

HCl: Hydrochloric Acid

mg/kg: Milligram/kilogram

ip: Intraperitoneal

LD_{50}: Lethal Dose 50

b.w: Body Weight

CCSEA: Committee for Control and Supervision of Experiments on Animals

(g): Gram

SGPT: Serum Glutamic Pyruvic Transaminase

SGOT: Serum glutamic-oxaloacetic transaminase

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