Indian J. Innovations Dev., Vol. 1, No. 1 (Jan 2012) ISSN 2277 - 5390

Effect of *Semecarpus anacardium* extract on physiology of brain in albino rat

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Abstract

Semecarpus anacardium Linn. is a medicinal plant known for its potent medicinal properties, used in Ayurveda and Sidhha, also popularly used in tribal and rural areas of India for treatment of various disorders and gaining immunity. It is known as 'rasayana' and reported in ancient literature that indiscriminate use of nut oil cause ill effects on skin, and eyes. *S. anacardium* pericarp oil extract (SAE) has commercial importance in preparation of herbal medicines and dyes. Large number of labors from rural areas are involved in extraction of oil, were found suffering from deformities due to exposure. No work was reported yet regarding toxic effects of *Semecarpus anacardium*. The present work deals with the assessment of effect of SAE on biochemical parameters (Total proteins, glucose, glycogen, cholesterol and Mg) and activity of GOT, GPT, LDH, SDH and AChE enzymes of brain of albino rat. Experimental albino rat after oral treatment of SAE showed alteration in studied parameters indicating the adverse effect on brain compared to control group. Outputs of the study signify the need of safe environment, safer extraction process and a proper training to those workers.

***Keywords*:** *Semecarpus anacardium;* Albino rat; Brain parameters; Toxicity; Environment safety.

Introduction

Semecarpus anacardium plant (family: *Anacardiaceae*) mostly found in tropical areas; is popularly known as 'dhobi's marking nut', in sanskrit called *Bhallataka/agnimukh*. It is important medicinal plant well known for it's potent medicinal properties and also have commercial importance. *S. anacardium* is commonly used in tribal communities of Maharashtra, Madhya Pradesh, Andhra Pradesh and Southern part of India for maintaining health and immunity (Choudhari & Deshmukh, 2012). In ancient Indian system of medicine Ayurveda and Siddha, in Unani as well as in Homeopathy *Semecarpus anacardium* used in the treatment of various diseases. Kernel from nut is nutritious. The anti-inflammatory and anti cancer properties of *Semecarpus anacardium* Pericarp oil obtained from nut is used in treatment of asthama, digestive disorders (Satyavati, 1969; Hembree, 1978; Premalatha, 2000). Nut oil is obtained by burning *S. anacardium* nut for consumption. Few drops of pericarp oil mixed in milk or ghee or vegetable oil were consumed for treating asthama, weakness. It is also applied on foot to eliminate pain. The dose intake was at random and indiscriminate (Choudhari & Deshmukh, 2006). *S. anacarddium* is hot in action. As per literature improper, irrelevant and indiscriminate use of *Semecarpus anacardium* create many serious condition such as red patches on skin, rash, sensitivity to heat and sunlight and needs careful intake and as per the suggestion and under supervision of physician/ experts (Desai, 1927; Nadkarni, 1993; Sastri, 1995; Gogate, 1997) [Photograph 1, 2, 3].

Pericarp oil contains flavonoids, phenols like catachol and anacardic acid as active constituents (Kirtikar & Basu, 1975; Premalatha, 2000) causing corrosive action. Tripathi *et al* (2008) suggested for purification of nut oil for the use in arthritis, but it appears to be inconvenient in large preparation at large scale. Pericarp oil obtained from nuts is also used commercially in preparation of dyes, medicines and as lubricants. For that huge amount of pericarp oil is required.

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Photograph 1,2,3

As per survey extraction of pericarp oil from nut is tedious, laborious process, in this work large number of labors mostly from rural areas are involved, they are exposed to pericarp oil, its fumes in nearby environment that create toxic effects like corrosive action, serious problems of skin, deformities in human/ labor exposed to *s. anacardium* nut oil during extraction [Photograph 4,5,6,7].

As no toxicity study was reported regarding *Semecarpus anacardium*, therefore, a work was conducted on albino rats (Wistar strain) under major research project UGC, New Delhi [F.No.3-29/97 (SR-II)]. SAE was found to caused adverse effects on digestive physiology and some haematological aspects of albino rat and was found to develop serious pathological conditions cause *Semecarpus anacardium toxicosis* in albino rats Choudhari (2004), Choudhari & Deshmukh (2007a), Choudhari & Deshmukh (2007 b).

Present work is aimed to assess effects of SAE on some biochemical parameters (total proteins, glucose, glycogen, cholesterol, Mg content) and activity of some enzymes (GOT, GPT, SDH, LDH

Photograph 4,5,6,7

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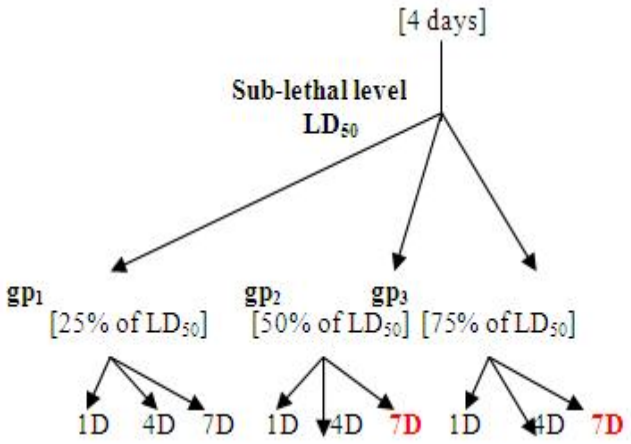

Experimental. Animal—Albino rat (*Wistar strain*), Sex—Male (M) & Female (F)
Animals/group—06, LD_{50} = 1gm /kg bw/ day
Shal level { 1D, 4D, 7D treatment}
gp_1—Lower concentration group = 250mg /kg b w/day
gp_2_ Medium Concentration group = 500 /kg b w/day
gp_3_ Higher concentration group = 750mg LD_{50} /kg b w/day
D stands for day or days
Observations were compared with control group animals

Fig. 1. toxicity study of SAE in Albino rat

and AChE) in brain of albino rat and intended to find support for need of safe method of extraction and safer environment to workers involved in extraction process.

Material and methods

The experiments were conducted in Toxicology Laboratory, Science College, Nanded. Ripen nuts of *Semecarpus anacardium* were collected from farm near Nanded city, were identified by Taxonomist from Botany Department.

Preparation of SAE

Pericarp oil was obtained by burning the crushed nuts on flame. *S. anacardium* extract (SAE) (50% w/v) was prepared in ground nut oil as vehicle.

Experimental animals

For experimentation, albino rats (Wistar strain) were used. Animals were procured from NIN, Hyderabad. Animal handling, experimental plan was approved by Institutional Animal Ethics Committee (IAEC), and by CPCSEA, 2000). Animals were provided with balanced diet prescribed by NIN, Hyderabad and kept *ad libitum* with 12 hrs dark and light cycles.

LD_{50} was determined as method by Litchfield & Wilcoxon (1949). The study was conducted at sub lethal level to find acute effects as in Fig.1. Animals were exposed to SAE orally along with balanced diet.

Parameters for study

a) Biochemical parameters: 1.Total proteins, 2. glucose, 3. glycogen, 4. cholesterol and 5. Mg contents. Total protein was determined by Biuret method as described by Oser (1965). Glucose content of brain was determined by using Anthrone reagent (Roe, 1954). Glycogen was determined by same method as for glucose. While glycogen content was calculated by multiplying glucose values by 0.927. Cholesterol content was determined by Zlatkis, *et al* method as described by Sood (1994). Magnesium (Mg) content was determined by modified Titan yellow method (Oser, 1965).

b) Enzymes: Activity of following enzymes from brain

1. GOT (*Glutamate Oxaloacetate Transaminase*)/AsT (*Aspartate Amino Transferase*) [EC2.6.1.1],
2. GPT *(Glutamate Pyruvate Transaminase*)/AlT (*Alanine Amino Transferase*) [EC 2.6.1.2]
3. LDH *(Lactate Dehydrogenase* [EC 1.1.1.27]
4. SDH (*Succinate Dehydrogenase*) [EC 1.3.99.1] and
5. AChE (*Acetyl cholinesterase*) [EC 3.1.1.7]

GOT /AsT and 2) GPT /AlT were determined by the method of *Reitman and Frankel* (Oser, 1965). Activity of 3) SDH was determine by Kun and Abood method as described by Nagbhushanam *et al*., (1981) and 4) LDH was determined by *modified Kun and bood method* as in Nagbhushanam *et al*. (1981). Activity of AChE was determined by biochemical method as mentioned by Varley (1976).

After desired period of exposure to SAE, animals were anaesthetized (Raghuramulu *et al.,* (1983) dissected and brain samples were collected. They were washed with water and soaked with tissue paper. 1% of aqueous homogenate was

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prepares for each animal in homogenizer. Homogenate was used for determination of alteration in selected biochemical and enzymes parameters.

Statistical analysis

Values are expressed as mean ± SD for (n=6) in each group of male and females. Significance of the difference was determined by Student's 't' test. Values with ($p<0.05$) as significant @, ($P<0.01$) as moderately significant # and ($p<0.001$) as highly significant (Raghuramalu, 1983).

Results

After 7 days of SAE treatment of animals of gp_2 and gp_3 showed 100% mortality during experiment. Observations were compared with control group. Results obtained for biochemical parameters are shown in Table 1. Result showed there were significantly increased proteins; increase was significant during 1 to 4 days. It was decreased after 7 days. Significant elevation in magnesium content was noted during 1to 7 days, while increase was insignificant after 1day treatment of lower dose in brain tissue of male and female. While significant decreased level of glucose and glycogen as well as cholesterol level were noted in brain tissue in experimental rats (Choudhari & Deshmukh, 2010a).

Experimental animals also showed prominent and significantly decreased levels of glucose in female albino rats. Effect on activity of enzymes were shown by histogram as in Fig. 2 (1, 2, 3, 4, 5) After oral treatment of SAE doses, significant increase of GOT, GPT, SDH, LDH and AChE activity was observed in brain of experimental animals (Choudhari & Deshmukh, 2010 b). A trend of increase of GOT activity was noticed in all groups, increase was more in female than males. Fall was seen after 1 day in male but it was insignificant. A trend of increased GPT activity was found in all survived group, GOT activity was in lower and middle concentration dose treated group, in male after 4 days it was insignificant and in female activity was insignificant after 1 day, while remaining group showed significant raised GPT activity. While LDH activity it was significant after 4 days in all treated groups, while after 1 day, significantly raised LDH activity was noted in females treated with higher (gp_3) and middle concentration dose (gp_2). Activity of SDH showed trend of elevated activity. The increase was insignificant in male and females of gp_1 after 1 day. Groups exposed to lower concentration dose (gp_1)

Table 1. Effect of Semecarpus anacardium Extract on Biochemical parameters in brain of albino rat

Day/ Group & Sex	**Total protein** [mg/gwetwt]	**Glucose** [mg/100gwetwt]	**Glycogen** [mg/100gwetwt]	**Cholesterol** [ml/DL]	**Mg content** [mg/dl]
0D M	33 ± 3.57	53.779 ±18.72	49.85 ±16.7	183.015 ± 10.48	1.403 ± 0.939
/Con F	34 ± 0.47	58.26 ±8.51	54.01 ± 8.45	187.951 ± 4.214	1.29 ± 0.74
1D/ gp_1 M	105.33 ± 9.54*	47.34 ± 13.02 I	43.88 ± 12.34 I	112.75 ± 3.53*	2.399 ± 0.81 I
F	80..0 ± 5.36 *	25.823 ± 1.58 #	23.63 ± 14.86 @	113.82 ±3.542*	2.674 ± 0.62 I
1D/gp_2 M	100.66±2.37 *	47.045 ±14.08I	43.61 ± 2.34 I	106.32 ±9.856*	2.52 ± 0.59 @
F	63.33 ±6.558 *	38.09 ± 16.77 @	35.3 ± 2.924 I	122.57 ±2.93*	2.63 ± 0.81 @
1D/gp_3 M	78.66 ± 2.379*	34.45 ± 9.02 I	31.54 ± 10.45 I	94.52 ± 4.10 *	4.29 ± 0.009 *
F	86.00 ±3.571 *	18.21 ± 12.49 *	16.87 ± 11.51*	85.047±9.16 *	3.33 ±1.45 *
4D/gp_1 M	121.33± 4.17 *	40.845 ± 16.03 I	37.89 ±14.84 I	117.045±2.44 *	4.81 ± 1.41 #
F	105.33 ± 4.76*	16.24 ± 20.54 #	15.06 ±19.05 @	59.79 ± 9.25 *	2.65 ± 0.268 *
4D/ gp_2 M	126 ± 3.57 *	34.28 ± 0.76 I	31.94 ± 10.45I	65.93 ±4.91 *	3.347 ± 0.47*
F	57.00 ± 5.366*	31.418 ± 2.4 @	31.95 ± 5.86 #	91.45 ±6.87 *	3.75 ± 0.47 #
4D/ gp_3 M	80.0 ± 3.57 *	20.45 ± 12.99 @	18.45 ± 12.13@	55.95 ±5.23*	3.378 ±1.55 @
F	64.00 ± 5.36 *	31.93 ± 4.51 *	29.69 ± 4.00 *	64.55 ±4.52 *	3.36 ± 0.91@
7D/gp_1 M	57.33 ± 4.76 *	11.20 ± 10.04 #	10.385 ± 9.28@	49.19 ±6.86 *	3.5 ± 0.67 #
F	53.33 ± 15.5 *	14.0 ± 14.53 *	12.98 ± 9.52 *	51.10 ±6.87 *	2.63 ± 0.54 @
7D/ gp_2 M	---	---	---	---	---
F					
7D/gp_3 M	---	---	---	---	---
F					

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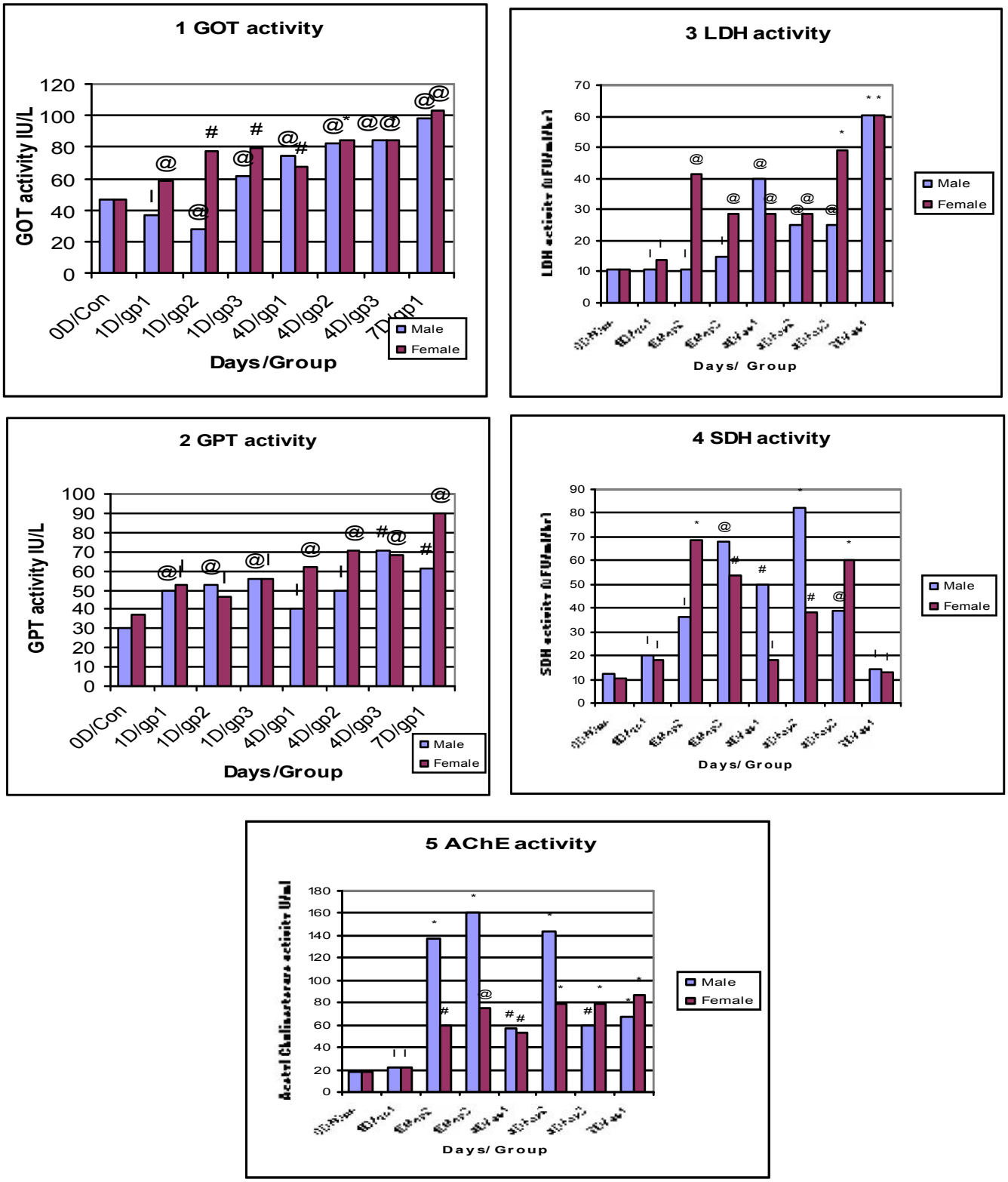

Fig. 2. Effect of Semecarpus anacardium extract on activity of enzymes in brain of albino rat

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Showed significant increased AChE activity after 4 and 7 days, while groups treated with higher doses showed significantly raised AChE after 1 and 4 days.

Discussion

In the present study, elevation of protein level may be as a result of increased permeability of the blood barrier due to inflammation and necrosis, and may be due to sclerosis indicating cerebral hemorrhage and release of smaller molecules of albumin and globulin. It also indicate impaired neurogenesis, may lead to conditions like Alzheimer's disease (AD). Increased total protein level also signifies the state of cerebral thrombosis and development of brain tumor (Cantarow & Trumper, 1949; Cella & Watson, 2000; Widmann, 1984). Development of a tumor like growth was reported in head region of exposed animals by Choudhari (2004). As per Muppala (2010), buildup of some toxic proteins in neurons causes neuron to malfunction and eventually to die.

Choudhari (2004) reported weak, decreased consumption of balanced diet, loss in body weight, adverse effect on carbohydrate, protein and lipid digestive enzymes in exposed animals; it is supported by Pandya (2010). In present study, significant fall in glucose indicated higher glucose consumption, as brain the organ that almost entirely run on sugar, it use almost 60-70% of glucose in taken that is consumed during increased metabolism after that there is fall in glucose level, indicating the state of malnutrition Therefore, to compensate the need of body and to gain energy, consumption of glucose was appeared as increased due to which animals showed decreased level of glucose, followed by fall in glycogen content in brain (Murray, 1990; Varley 1976).

Highly significant fall in cholesterol content indicate the state of hypo cholesterolemia developed in brain of experimental animals. That also indicate liver dysfunction, due to liver damage to greater extent, and that is also a sign of liver cirrhosis in experimental animals, as in body cholesterol is produced by liver. It may be due to increased level of phospholipids as well as lipoprotein level. Thus in animals fall in cholesterol level may lead to condition of AD Alzheimer's Disease (Widmann, 1984).

Rise in Mg content may also be to compensate lowered level of Ca and other electrolyte levels in SAE treated rats. Elevated level of Magnesium appears to play important role in stabilizing the brain wave pattern and to increase blood flow to brain. Increased Mg content is usually found in severe hepato-renal damage and when protein level is elevated.

A prominent trend of increased in GOT, GPT, LDH, SDH and AChE activities observed were correlated with severe tissue damage and leading to necrosis and release of enzymes in brain tissue in albino rats exposed to SAE doses. Results revealed that administration of SAE cause serious injury brain tissue that also indicated imbalance in CNS and neuromuscular system (Cella & Watson, 2000) appears developed pathological conditions in experimental animals. Similar changes of tissue damage were seen in other vital organ liver, kidney, pancreas, duodenum of treated animals Choudhari (2004).

Observations were supported by work of Wolf & William (1973), the elevated GOT, GPT, LDH activity were found due to tissue damage and later necrosis of brain tissue as well as spinal cord, which was damaged to 26-30 %, with release of enzymes. Elevation may be due to necrosis of brain due to cerebral thrombosis and cerebral hemorrhage resulted in elevation of GOT as in case of myocardial infarction. In case of cerebral infarction following cerebral thrombosis or cerebral hemorrhage, develop an increase in GOT and LDH_2 and LDH_3 enzymes. An increased SDH activity and LDH activities in all survived groups is a sign of necrosis (Bergmeyar, 1980; Cella & Watson, 2000). Regarding increased AChE in brain and in blood plasma indicated a sign of acute myocardial infarction in rats (http://enz wilkipedia/cholinesterase). This may be the one of the possible reason for 100 % death of treated animals of gp_2 and gp_3 after 7 days. In our study AChE activity was elevated in brain tissue that indicate cellular tissue damage after exposure in brain.

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As per Murugesen *et al.* (1999) the activity levels of GOT, GPT was increased in brain suggested that increased glucogenesis to satisfy the energy demand of brain after carbaryl toxicity. As per the study of Villa (1981), changes with maximal rate of enzymatic activity of LDH, GOT, AChE related to neurotransmission in case of pure and crude fraction of brain of rat. As well as in cerebral cortex, author suggested that the observed activity certainly be the expression of disturbed metabolism neurons and brain physiology,

As compared to control, a prominent and significant change of elevation in activity of GOT, GPT, LDH, SDH and AChE, indicated severe injury to nervous system were correlated with changes behavioral changes, paralytic symptoms, convultions observed during the study (Choudhari, 2004; Duffus & Worth, 1996).

Conclusion

Exposure of animals to SAE showed ill effects on brain parameters; ultimately on physiology and function of brain. Therefore, outcome of present study can be useful as measure for neuro toxicity and disturbed function of Central Nervous System (CNS) in treated albino rats after sub lethal dose treatment of SAE. Results also signify further work is essential in this regard. Hence, the outputs of present study, direct us in finding the way for the safety of workers involved with urgency, in order to avoid improper environmental conditions causing hazard at work place, it also compel us to think about method which provide safety as well as proper education and training to workers which are involved in separation of kernel and extraction of pericarp oil from *Semecarpus anacardium* nuts to avoid exposure. We direct further research work in that line.

Acknowledgement

Authors are thankful to UGC, New Delhi for finance to our major project. They are also grateful to Principal, NES's Science College, NANDED for constant encouragement.

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