

VIII. FOOD & DRUG TOXICOLOGY RESEARCH CENTRE

A. FOOD SAFETY

1. OCCURRENCE OF TRANSGENIC MATERIAL IN DIFFERENT VARIETIES OF BT COTTON

The genetically modified BT cotton that is currently being cultivated and marketed in India carries Cry 1AC gene which expresses insect resistance protein. BT cotton may enter the food chain as feed for cattle or as edible oil. This study was initiated to detect presence of transgenic DNA and its protein in cotton seed and oil.

Aims and objectives

Detection of transgenic DNA and protein (Cry1Ac) in cotton seed samples from farmers.

Work done during the year

1. Collection of samples

A total of 10 BT cottonseed samples, 4 cottonseed extractions and 4 cottonseed oil samples were collected from farmers and oil extraction plants in Warangal and Guntur districts. Authentic BT cottonseed samples namely, MECH 12, 162 and 184 were procured from MAHYCO Company for comparison as standard.

2. Analysis of BT cottonseed for Cry1Ac delta endotoxin

BT cottonseed samples (10) along with the standard BT cottonseed samples were analyzed for Cry 1Ac using commercial kit manufactured by Envirologix Inc. USA. The kit method is based on ELISA (Sandwich format) with primary antibody coated wells to which a clarified protein extract of sample is applied.

After reaction with Cry1Ac enzyme conjugate, the absorbance is read at 450nm. Cry1Ac level is calculated on the basis of OD values of standard /calibrators (provided with the kit) and samples. The results indicated that Cry1Ac levels ranged from 0.35 to 1.92 ppm in samples collected from farmers and 1.46-1.77 ppm in the MAHYCO BT samples MECH 12, 162, 184.

Conclusions

Presence of Cry 1AC protein was detected in samples collected from the farmers. The levels were comparable to that of standard seed samples obtained from MAHYCO.

2. EFFECT OF DIFFERENT TYPES OF DOMESTIC PROCESSING ON PHENOLIC CONTENT AND AOA OF SELECTED FOODS

Plant foods are good sources of antioxidants. Several studies have reported antioxidant content of various foods, mostly in their raw form. However, during processing, interactions among nutrients and/or antioxidants and/or oxidants, may modify the antioxidant activity of foods. Therefore, information on antioxidant activity of foods, in the form they are consumed is useful. Hence, studies have been undertaken to determine antioxidant activity of foods, both in the raw and processed forms. This study is an attempt to determine the effect of processing on the antioxidant activity (AOA) of foods, and to use the data thus generated, for the development of suitable recipes with higher antioxidant activity.

Aims and Objectives

To determine the effect of different types of domestic processing on phenolic content (PC) and AOA of selected foods of different categories.

Work done during 2004-2005

Keeping in view that most plant foods are usually consumed after some kind of processing or the other, plant foods belonging to various categories, which were found to have higher AOA in raw form (Annual Report 2003-2004) were selected to study the effect of different types of domestic processing on their AOA and PC. The foods selected to study the effect of domestic processing were: wheat (cereals), red gram dhal and black gram dhal (dehusked legumes), green gram, Bengal gram and moth beans (whole legumes), groundnut and sesame (oil seeds), spinach (green leafy vegetables), tomato (other vegetables) and onion (roots and tubers). As mentioned earlier (Annual Report 2003-2004), three samples of each of these foods were purchased from each of the three selected local markets. They were cleaned thoroughly and subjected to commonly used domestic processes such as boiling in water, pressure-cooking, shallow frying, deep-frying, sprouting, malting and microwave cooking appropriately. The raw and processed food samples were extracted with 70% methanol, the AOA and phenolic content were determined in these extracts as mentioned earlier.

Results

The results are given in Tables 22 and 23 and the salient findings of the study are mentioned below.

Antioxidant Activity

- During sprouting and malting there was an increase in AOA of wheat albeit, the increase was not significant. In general, other processing methods studied had no significant effect on the AOA of wheat.
- AOA decreased significantly during deep-frying of soaked black gram dhal ($P < 0.05$). Other types of processing had no significant effect on the AOA of dehusked legumes.
- Sprouting had no significant effect on the AOA of whole legumes.
- There was no significant difference in the AOA of raw and processed vegetables (GLVs, other vegetables, roots and tubers) and oil seeds.

Keeping in view our earlier finding that PC could account for the AOA in only some types of foods but not others, we determined the effect of processing on the PC of foods and evaluated the relationship between the AOA and PC in the processed food. The salient findings on the PC of processed foods are as given in table 22.

Table 22. Effect of domestic processing of Cereals and Legumes on AOA & PC

FOOD STUFF		Process	AOA*	PC**
Cereals	Wheat	RAW	5.8 ±2.7	24.0±5.5
		BOIL	5.3 ±2.8	18.1±4.6
		PR.COOK	4.4 ±0.6	13.5±2.6
		PR.C.SEP	4.7± 1.5	15.5±4.2
		R+ OIL	4.9 ±1.4	26.0±7.1
		SH.FRY	4.6 ±1.3	24.5±6.7
		PURI /sf	5.6 ±2.1	25.8±3.0
		ROTI	4.7 ±2.1	31.0±9.6
		DR	5.4 ±1.8	30.6±2.6
		SPROUT	3.8 ±0.5	56.6±17.0
MALT	3.8 ±2.0	101.2±20.8		
Dehusked legumes	Black gram dal	RAW	1.2± 0.2	26.1±0.7
		PR.COOK	1.8 ±0.1	22.6± 2.0
		PURI /sf	2.6 ±0.8	26.2± 2.8
		DR	1.8 ±0.1	36.3± 3.6
	Red gram dal	RAW	1.5 ±0.3	24.9± 7.9
		BOIL	1.3 ±0.5	27.2± 5.2
		PR.COOK	1.9 ±0.5	23.4± 5.8
		PR.C.SEP	1.5 ±0.4	28.7± 9.4
		R+ OIL	0.8 ±0.2	24.4± 1.1
		SH.FRY	2.5 ±1.4	32.5± 8.8
DR	2.0 ±0.9	34.9± 5.6		
Whole legumes	Bengal gram whole	RAW	0.8 ±0.3	36.0± 11.8
		SPROUT	1.0 ±0.1	40.6± 5.5
	Green gram whole	RAW	0.8 ±0.1	69.4± 3.7
		SPROUT	0.9 ±0.2	100.9± 6.1
	Moth	RAW	1.0 ±0.1	34.8± 2.9
		SPROUT	0.8 ±0.1	186.6± 2.9

*AOA expressed in mg of raw food required for 50% inhibition of auto-oxidation of β carotene - linoleic acid emulsion under the conditions of the assay.

** PC expressed in mg of gallic acid equivalent in 100g of raw food stuff

Phenolic content

- During sprouting and malting there was a significant increase in phenolic content of wheat. Other processing methods had no significant effect on PC of wheat.
- Phenolic content of dehusked legumes increased significantly only during dry roasting but no change was observed on other types of processing.
- Sprouting in general increased the PC of whole legumes but the effect varied with different legumes.
- PC of spinach was significantly increased during boiling ($P<0.05$) and pressure-cooking ($P<0.05$). Other processing methods also increased the PC of spinach but the increase was not significant. No significant changes were observed in PC of tomato and onion during any type of processing studied.
- Groundnut and sesame had the highest amount of PC among all the foods studied. However there was no significant effect of any of the processing methods tested on their PC.

Table 23. Effect of domestic processing of vegetables and oilseeds on AOA & PC*

FOOD STUFF		Process	AOA*	PC**	
Vegetables	Spinach	RAW	3.2 ±1.5	33.8± 7.3	
		BOIL	2.9 ±1.5	53.6± 10.2	
		PR.COOK	3.4 ±1.7	51.8± 9.6	
		PR.C.SEP	2.5 ±1.7	42.7± 8.0	
		R+ OIL	4.3 ±2.2	30.4± 5.0	
		SH.FRY	3.4 ±1.5	48.2± 10.6	
			Micro	3.2 ±1.7	47.3± 9.2
	Tomato	RAW	3.6 ±1.6	35.9± 3.4	
		BOIL	4.3 ±1.8	36.8± 2.6	
		PR.COOK	3.5 ±0.8	35.7± 4.1	
		PR.C.SEP	3.3 ±1.0	35.8± 2.7	
		R+ OIL	3.1 ±1.1	32.7± 6.9	
SH.FRY		4.1 ±1.2	39.1± 1.9		
		Micro	4.0 ±1.2	36.9± 5.0	
Onion	RAW	4.2 ±1.2	39.2± 6.1		
	BOIL	5.6 ±1.7	32.7± 2.2		
	PR.COOK	5.0± 2.0	31.3± 4.1		
	PR.C.SEP	4.8±1.7	32.0± 3.2		
	R+ OIL	3.8±1.4	43.9± 3.7		
	SH.FRY	4.9±1.3	45.2± 11.8		
		Micro	4.8±1.9	36.0± 5.8	
Oil seeds	Groundnut	RAW	2.3±0.9	95.8± 15.6	
		BOIL	2.6±1.9	99.7± 47.6	
		PR.COOK	2.4±1.6	105.7± 25.2	
		PR.C.SEP	2.0±1.2	127.9± 31.4	
		R+ OIL	1.8±1.5	125.8± 21.0	
		SH.FRY	2.6±1.4	116.0± 20.6	
			DR	3.0±1.2	94.9± 19.5
	Sesame	RAW	3.7±0.3	89.1± 14.4	
		DR	3.4±0.2	89.2± 17.1	

*AOA expressed in mg of raw food required for 50% inhibition of auto-oxidation of β carotene - linoleic acid emulsion under the conditions of the assay.

** PC expressed in mg of gallic acid equivalent in 100g of raw food stuff

In line with the observation we made earlier in raw foods, the AOA and PC of processed foods also do not seem to go hand in hand always and in all foods. Also, the effect of processing could be different on the AOA and PC of not only foods of different groups but also among foods of same groups as well. In general, all the processing methods tested, either had very marginal or no effect on their AOA. Among different types of domestic methods of processing studied PC seemed to increase during sprouting but the increase varied among different legumes. Sprouted legumes had the highest AOA amongst all the processed foods tested.

B. CANCER AND XENOBIOTICS

1. ANTIGENOTOXIC POTENTIAL OF GINGER

Some frequently consumed spices in India and other countries have been claimed to exhibit antimutagenic/anticarcinogenic potentials. The antimutagenic property of ginger was reported in previous report (Ann Rep, NIN 2003-2004). One of the suggested mechanisms of antimutagenicity is through scavenging of free radicals that are generated during xenobiotic metabolism. The antioxidant enzymes in the tissues effectively counteract the endogenously formed free radicals. Nutrients and non-nutrients in diet are known to possess antioxidant property and also stimulate antioxidant enzymes in tissues. Therefore an experiment was conducted to investigate, whether ginger fed through diet can improve the antioxidant status in experimental animals.

Rats were fed ad lib with ginger through diet at various levels namely 0.5%, 1%, 5% for a period of one month. The animals were sacrificed and organs of interest namely liver and kidney tissues were dissected out and processed for antioxidant enzymes.

Results

In the previous Ann Rep (2003-2004) results on TBARS and protein carbonyls were reported. It was found that there was significant reduction in the levels of TBARS in liver and kidney. The level of protein carbonyls in liver was decreased in 5% ginger fed groups. Hepatic superoxide dismutase, catalase and glutathione peroxidase activities were measured. The results indicated significantly higher activity of liver SOD, catalase and GSHPx at all the levels of ginger feeding ($P < 0.001$) compared to control (Table 24).

Table 24. Hepatic antioxidant enzymes in ginger fed rats

Group	SOD U/mg. prot.	Catalase U/mg prot.	GSHPx	
			Cytosol oxidized/ mg.prot per min	RBC U/gm Hb
Control	2.9 ± 0.48 ^a	40.4 ± 3.47 ^a	332.9 ± 38.64 ^a	267.0 ± 35.1 ^a
0.5% ginger	5.1 ± 1.21 ^b	55.4 ± 10.06 ^b	370.1 ± 29.90 ^b	312.0 ± 29.2 ^b
1.0% ginger	6.1 ± 0.89 ^c	66.7 ± 7.87 ^c	401.3 ± 27.62 ^b	327.7 ± 6.78 ^b
5% ginger	7.0 ± 0.89 ^{bc}	78.4 ± 2.72 ^d	433.3 ± 23.86 ^c	379.5 ± 25.8 ^c

Values are Mean ± SD of 6 rats per group

Different superscripts are significant at $p < 0.05$ by Duncan's multiple range test

Conclusion

Ginger ingestion can result in improved antioxidant status and this could be one of the bases for its antimutagenic property.

2. ETHNOPHARMACOLOGICAL VALIDATION OF BIODYNAMIC COMPOUNDS IN TRADITIONAL MEDICINE

Natural and synthetic antioxidants play a vital role in protecting cells and tissues against oxidative damage caused by free radicals. Previous studies (Annual Report-2002) indicated that plant extracts coded 4308,4212,3107,3223 & 5322 have potential antioxidant activity as evaluated by battery of *in vitro* tests. Further, the therapeutic potential of plant extracts 4212, 3223 was evaluated in patients suffering with rheumatoid arthritis and found co-relation between the therapeutic efficacy and the antioxidant potential (Annual Report-2003).

Explant culture system, an ex-vivo testing tool, a classic model for studying pharmacological, toxicological and drug metabolizing studies was used to confirm antioxidant potential of 4212 and 4308 extracts.

Hypothesis

To assess the antioxidant potential of aqueous (4212) and aqueous alcoholic (4308) extract of "Rasna panchaka" a combination medicine for the treatment of rheumatoid arthritis using a novel serum free explant culture system.

Methodology

Mice liver was excised aseptically, cut explanted to 1-2mm³ (1-2 mg each) cubes and cultured in Serum Free Medium 199 (Sigma) at pH 7.4 under aseptic conditions. In each petri plate, a fixed number (12-15) of explants were maintained in 5ml of medium 199 in a humidified CO₂ incubator at 37°C. Extracts varying from 1-10µg/ml were added to the culture plates.

The antioxidant property was assessed by determining the primary defense oxidative enzymes viz. SOD, Catalase, GSH and MDA in the cultured mice liver explants.

Results

- 1) Cultured tissue harvested at various time points clearly indicated that the cellular architecture of the tissue was well conserved in the first 6hrs with gradual display of specific changes in the next 24hrs (Figure 38).
- 2) The extracts showed significant protection against oxidative stress at the dose of 2 µg/ml (Figure 39).
- 3) Lipid peroxidation measured using malonaldehyde (MDA) as a marker was reduced by 50%. This effect was accompanied with an increase in the first defense enzymes superoxide dismutase (50%) and catalase (18%) with no change in reduced glutathione levels (Figure 40).

Fig. 38. Photomicrographs of percent normal cells at various time points

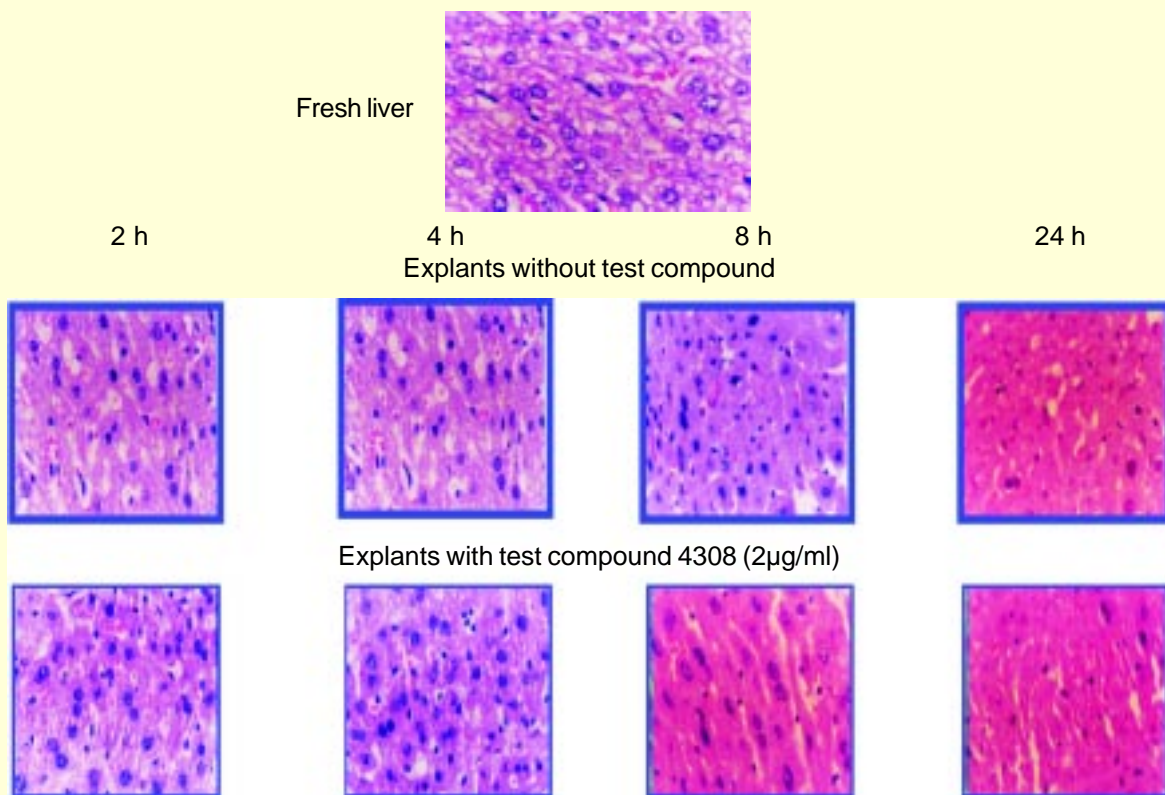


Fig. 39. Percentage of normal cells with reference to time and dose

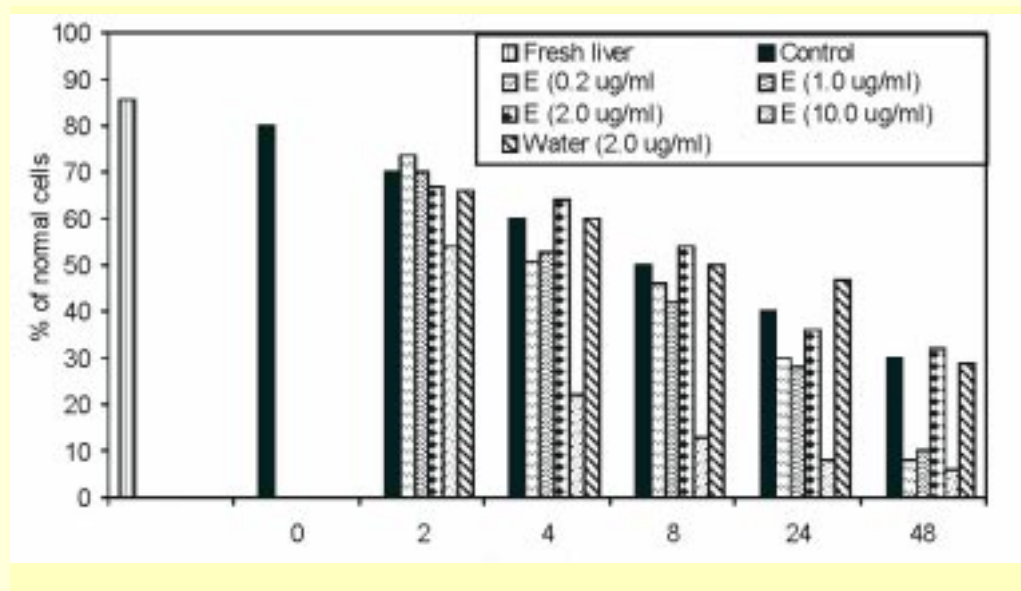
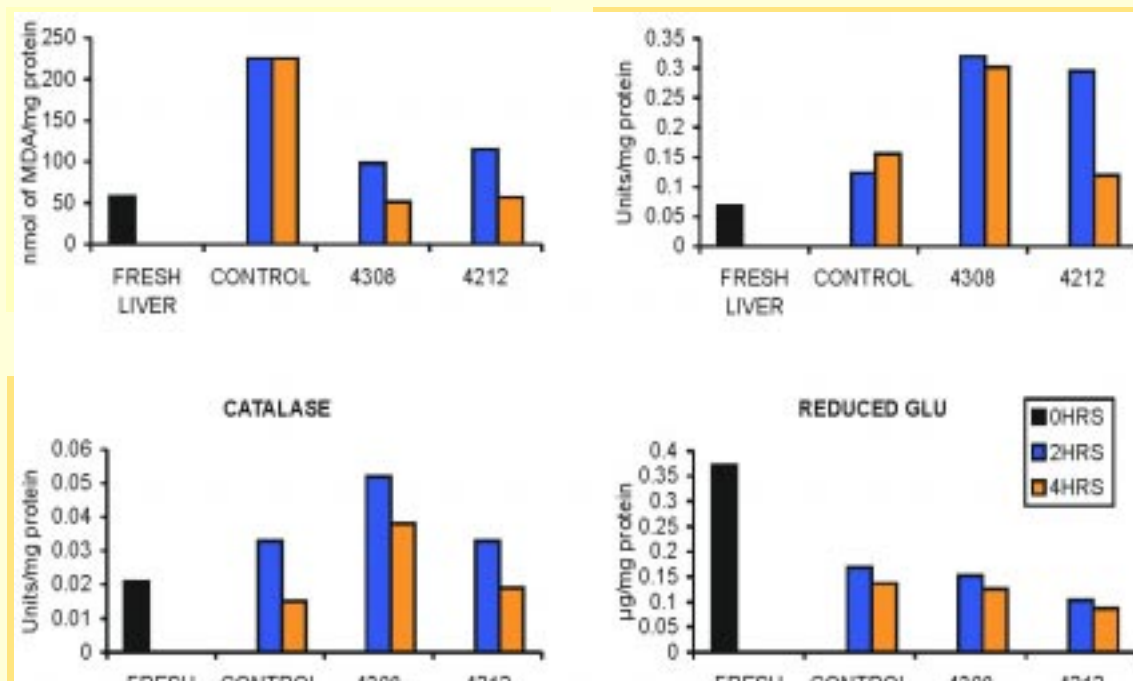


Fig. 40. Antioxidant status of explants with and without test compounds



Conclusions

- The study results related to antioxidant activity supported our earlier in vitro and in vivo observations validating the use of explant culture system to detect antioxidant potential of plant products.
- The results suggest that water plus methanol extract possess superior antioxidant property compared to the traditionally used water extracts.
- The results demonstrated that "Rasna panchaka" an indigenous drug used in the treatment of rheumatoid arthritis has a potential anti-oxidant property and can counteract the oxidative damage associated in the pathogenesis of disease.

3. IMPACT OF INTERVENTION PROGRAMME ON RATIONAL USE OF DRUGS (RUD). (PHASE - III)

In recent years, the use of drugs has considerably increased in almost all countries. Use of irrelevant and unnecessary drugs not only escalates the cost of health services but it is also harmful to the consumer under certain circumstances. Earlier studies conducted in various parts of Andhra Pradesh (Annual Report 2001/02) have indicated irrelevant use of drugs specially injectables, antibiotics, nutritional products etc. In a developing country like India with average literacy/socio-economic status it is imperative to initiate intervention studies to educate the public on the rational use of drugs. In view of this an attempt was made to develop intervention tools, which include film titled "Haridas - Tale of medicines", a brochure for creating awareness on concepts of rational drug use in community. A pilot interventional trial has shown a significant impact (Annual Report 2003-04).

The present study has been undertaken with the following objectives:

- To create awareness on the concepts of RUD with special emphasis on harmful effects of unnecessary use of injectables, antibiotics, nutritional product etc.
- To evaluate the impact of advocacy programme on RUD practices on target group.

Methodology

The study has been undertaken in a selected area in Karimnagar district of Andhra Pradesh. The baseline and post intervention data (after 2-3 weeks of advocacy programme) on drug consumption profile has been collected from 11 public health centres (7PHC, 3CHC & DH) which were selected based on the distinct socio-economic factors and from all directions in a pre-tested schedule. The intervention programme consists of a film show "Haridas - A tale of medicines" of 36 min followed by distribution of brochures at least twice in five days at health centers and public places. The data has been analyzed using various indicators viz. Prescribing, Patient care, Facility and Communication indicators as per WHO standard guidelines using SPSS package version 10.0.

Results

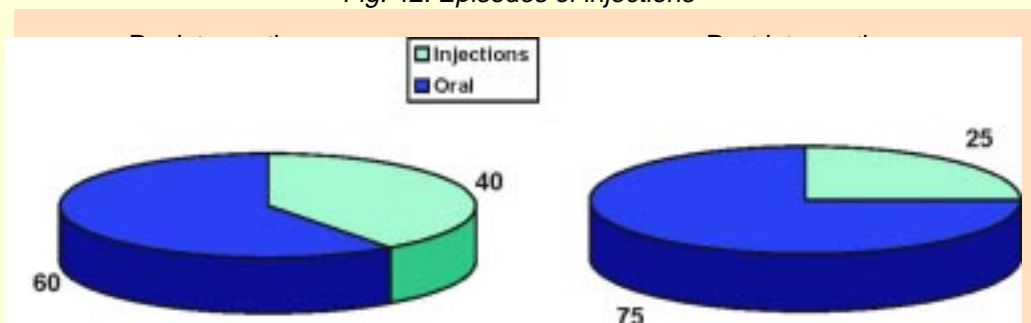
1. Approximately 50-100 patients of different age groups at health centers, about 150 community members during the show at public places/ schools/ panchayat office etc. have received the benefit of this intervention programme (Figure 41).

Fig. 41. Community education programme on rational use of drugs



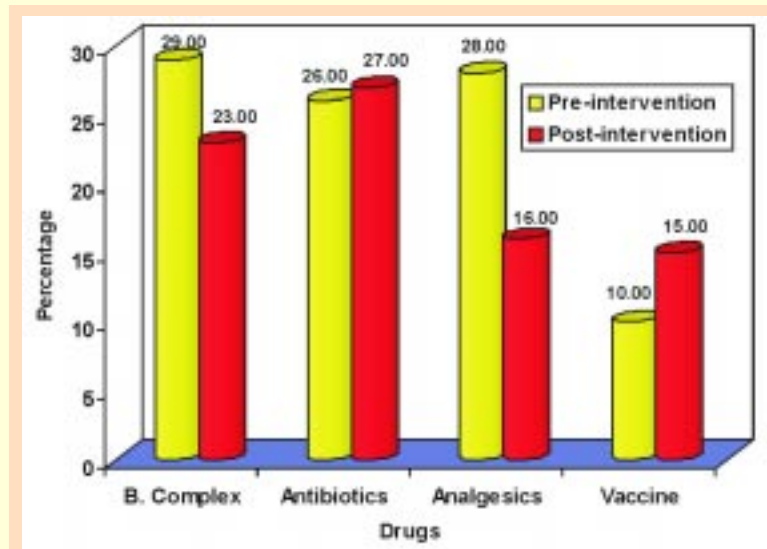
2. The various categories of the drugs prescribed and dispensed included analgesics (17%), vitamins (18%), antibiotics (10%) and other categories of drugs like anti-allergics, vaccines, topical applications etc (Figure 42).

Fig. 42. Episodes of injections



3. The use of injections was to the extent of 40%, for vitamins (22%), antibiotics (27%), analgesics (28%) etc.
4. The post intervention programme has shown a reduction in injection rate from 40 to 25%. (Figure 43).

Fig. 43. Categories of Parenteral Formulations Dispensed



5. A marginal group of population has clearly understood the importance of antibiotic dosage schedule.
6. The media coverage and encouraging support from medical and other officials has made a positive impact.

Conclusions

1. The study revealed excess use of injections (especially vitamins/ antibiotic formulations).
2. The interventional programme had a significant positive impact on target audience.
3. Good media coverage, active participation of community leaders and other officials especially from police and health departments have popularized the intervention programme in the community. There was a great demand for such programmes in other areas too.

The well-devised IEC programme can effectively promote RUD concept especially among under privileged sections of population.